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## *Contents*



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# THE FORM OF BEAT IN CILIA OF *STENTOR* AND *OPALINA*

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(*Received* 26 *May* 1959)

(With Plate 1)

## INTRODUCTION

Descriptions of the form of beat of cilia seem to be nearly as diverse as the sources from which the cilia are taken, although some features are common to most descriptions. Typical features are shown in Gray's (1922) diagrams of the movements of a frontal cilium of *Mytilus*. The beating cycle is divided into two distinct movements, a rigid effective stroke and a flexible recovery stroke. The effective stroke is often more rapid than the recovery stroke, taking only one-fifth of the total cycle in *Mytilus* lateral cilia (Gray, 1930).

The internal structure of cilia is now well known (Fawcett & Porter, 1954 and Bradfield, 1955) and it is thought likely that the outer nine fibrils (or doublets of fibrils) described by these authors are responsible for the contractions of the cilia. Bradfield has based an explanation of the form of beat shown by *Mytilus* abfrontal cilia, as figured by Gray (1930), on a sequence of contractions in these fibrils. The same explanation does not seem directly applicable to flagella, which are known to have the same internal structure, and would be expected to behave in a similar way. A modified theory which could account for the movements of both cilia and flagella is desirable.

The peristomial cilia of *Stentor* are large compound cilia which beat rapidly and show a form of beat that is quite different from that of *Opalina* cilia, which are slender single cilia with a much slower beat. Comparison of the movements of these two types of cilia may give indications of the reasons for the diverse forms of beat that have been observed.

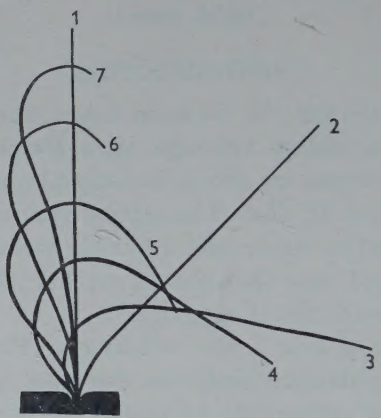
## OBSERVATIONS

### (*a*) *Stentor*

The beating of the peristomial cilia of *Stentor polymorphus* (Ehrbg.) has been observed under the stroboscopic illumination described previously (Sleigh, 1956, 1957); in which papers the arrangement of the peristome cilia was also described. These cilia beat in a plane at right angles to both the direction of the metachronal co-ordination and the peristome edge. The observed sequence of movements is shown in Text-fig. 1.

The effective phase of the beat occurs in stages 1 to 3, and is seen to be a swing of the bulk of the shaft of the cilium through up to 140°, correlated with a bend to the

right at the base. The recovery phase is shown in stages 3 through 7 to 1, and appears to result from the passage of a wave of flexure from the base of the cilium to the tip. It is significant that the point of maximum curvature has already started to move up the cilium before the full amplitude of swing has been achieved (stage 3). The two phases of beat in this cilium are thus not completely separable, as has been assumed in other cases, and the effective phase merges into the recovery phase. A single wave of flexure passing up the cilium from base to tip appears to be responsible for both phases of beat, causing the effective phase when the cilium bends at the base and the recovery phase as the bending wave progresses up the cilium.




Text-fig. 1. The sequence of movements of a peristomial cilium of *Stentor*.

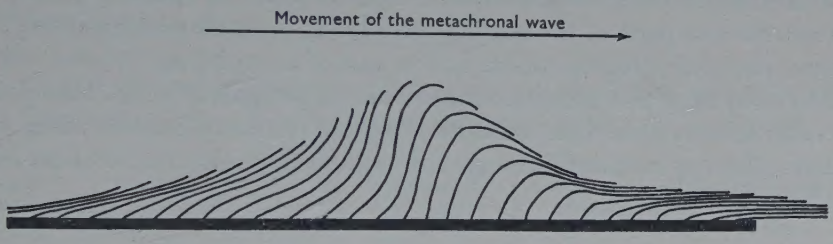
If we assume that a new flexure starts at the base when the previous one has reached the tip, which is consistent with the observed movements of these cilia, then the rate of propagation of the flexure can be obtained from frequency × length of cilium. This estimate will err on the low side, but cannot be far from correct since there is no appreciable interkinetic period. The average length of cilium was 30·4 $\mu$ (thirty observations), and the average frequency of beat at 18·5° C. was 27·7 beats/sec. (over 300 observations). Thus the average rate of propagation of the wave of flexure was 844 $\mu$/sec., with a range from 730 to 920 $\mu$/sec. at this temperature. These values are very close to the velocity of propagation of contraction waves found by Gray (1955) in sea-urchin sperm tails.

### (*b*) *Opalina*

The arrangement of cilia in opalinids is well known, both from studies under the light microscope (Konsuloff, 1922; ten Kate, 1927, and others) and under the electron microscope (Pitelka, 1956). These authors describe and figure a complete covering of body cilia which are arranged in rows. Pitelka has also described the fine structure of the cilia and their basal connexions.

The animal used in this investigation was *Opalina ranarum* (Ehrbg.), which was obtained from the rectum of the common frog *Rana temporaria*. Most of the recorded observations were made during January and February.

Examination of *Opalina* under dark-ground illumination shows obvious metachronal waves which normally pass backwards over both dorsal and ventral surfaces of the animal (see Pl. 1 A). This wave pattern is, however, very variable, and the direction of the metachronal wave transmission seems to change almost instantaneously to give a quite different pattern of waves (Pl. 1 B–D). This characteristic of metachronism in *Opalina* has been described by Okajima (1953), who has given a detailed account of the changes and has induced them electrically. Parducz (1954, 1958) has described a similar variability in the direction of the metachronal wave transmission in *Paramecium* as well as *Opalina*. The plane of ciliary beat in *Opalina* is at right angles to the crest of the metachronal wave, and the effective stroke is in the same direction as the metachronal co-ordination, so that the metachronism is symplectic (Knight-Jones, 1954). These cilia must be able to beat in any plane, the plane of beat varying with the direction of wave transmission.




Text-fig. 2. The component cilia of a metachronal wave of *Opalina*. Successive movements of a single cilium can be followed by moving along the row of cilia from right to left. The animal is moving towards the left.

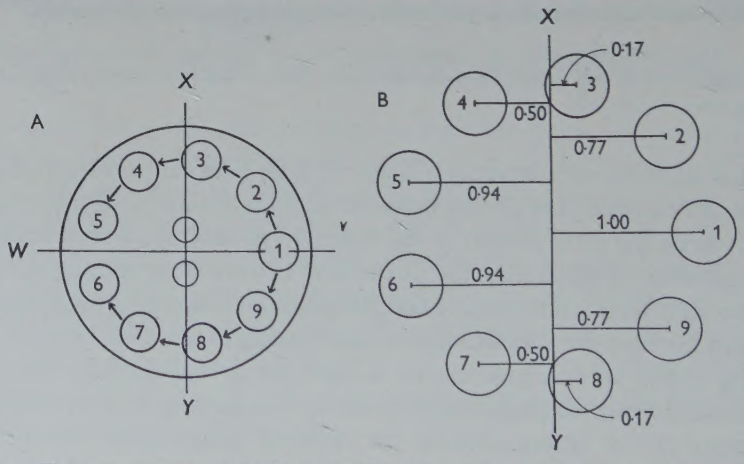
The form taken by cilia at various stages of their beat can be seen in Text-fig. 2, which was drawn from ciné photographs of the type shown in Pl. 1 E. The form of beat seems to be the same whatever the direction of beat; it appears identical both when the effective stroke is directed posteriorly in normal swimming and when the effective stroke is directed anteriorly and the animal is swimming backwards. At the beginning of the beat the cilia lie more or less parallel to the body surface, and there is a resting period in this position in at least the slower-beating cilia. The cilia straighten out during the preparatory phase by the passage of a flexure from base to tip, similar to that responsible for the recovery phase in the beat of a *Stentor* cilium. When this wave of flexure has travelled about one-half of the length of the cilium, a second bending wave, which pulls the cilium back towards the surface of the animal, appears at the base of the cilium and brings about the effective phase of the beat. The beating cycle is completed when the bending wave of the effective stroke has been propagated to the tip of the cilium. The whole of the beat takes place in one plane. It appears likely that movement of the whole metachronal wave serves to propel the animal through the water, rather than the movements of individual slender cilia.

Beating of *Opalina* cilia is less regular than that of *Stentor* cilia. The frequency of beat observed has varied between about 1 and 4 beats/sec. in different animals

at 17–18° C. It may also vary at different parts of the surface of a single animal, the posterior cilia beating more slowly than the anterior ones, in which case the successive metachronal waves coalesce as they move towards the posterior end. The rate of transmission of the bending wave along the cilia was measured from ciné photographs, and was found to vary between about 20 and 100 $\mu$/sec. The length of cilia of *Opalina* varied between about 10 and 20 $\mu$, depending on the size of the animal and the position on the body. The cilia shown in the photographs and drawings are about 15 $\mu$ long.

## DISCUSSION

The explanation of the cycle of contractions of cilia recently put forward by Bradfield (1955) will not explain the pattern of beat observed for cilia of either *Stentor* or *Opalina* without some modification. The ciliary beat in both animals involves the passage of flexures from the base of the cilium to the tip. Neither type of beat includes an effective phase that is separate from the recovery phase, for in both cases the two parts of the beating cycle are in progress simultaneously. It is suggested, therefore, that the whole beat is part of a continuous process, which is caused by a sequence of localized contractions in the peripheral fibrils. This assumes, as previous authors have done, that there is some system of cross-bonding within the cilium allowing localized bending of the cilium by contraction of short lengths of the internal fibrils.




Text-fig. 3A. The arrangement of fibrils in a cilium (from Bradfield (1955) and Fawcett & Porter (1954)). It is suggested that excitation of the peripheral fibrils takes place in the sequence shown by arrows, starting with fibril 1. B. The relative distances of the peripheral fibrils of a cilium from the line $XY$ (see text).

### (*a*) *Theoretical considerations*

An active ciliary flexure must result from a greater contraction of fibrils on the side of the cilium that is towards the centre of curvature than on the outer side of the bend. Thus if the contractile force in fibrils on the right of line $XY$ in Text-fig. 3A exceeds the force in fibrils on the left, the cilium will bend to the right, and vice versa. If fibrils are excited in the sequence shown by arrows in Text-fig. 3A,

and contractions are propagated up the fibrils from base to tip at the same time, then the contraction will have passed some way up fibril 1 before fibrils 2 and 9 have been excited, and the cilium will bend towards fibril 1. Continuation of contraction and excitation processes will result in the contractions of three and later five fibrils at one side of the ciliary base before any fibrils on the other side have been excited, so that the cilium will have bent further towards fibril 1. This flexure will pass up the cilium and, as the excitation spreads to fibrils 4 and 7, the contractions on the two sides of the cilium will become more balanced and the curvature of the cilium will be reduced. When all the fibrils have been excited, that part of the cilium in which all the fibrils are contracted will be straight, while the previous bending stages will be recognizable further up the cilium. Sooner or later the fibrils will relax, presumably in the order in which they contracted, so that as fibrils 1, 2 and 9 and 3 and 8 relax in succession, the cilium will bend in the opposite direction because the fibrillar contraction is unbalanced on the opposite side. The cilium will straighten out once more when all the fibrils are relaxed.

Table 1. *The sequence of bending moments about the line XY at successive stages of contraction, where F is the contraction force exerted by a single fibril*

| Stage of contraction | Fibrils contracted | Bending moment towards the right | Bending moment towards the left | Resultant bending moment and direction |
|---|---|---|---|---|
| A | 1 | 1·00*F* | — | 1·00*F* Right |
| B | 1; 2, 9 | 2·53*F* | — | 2·53*F* Right |
| C | 1; 2, 9; 3, 8 | 2·88*F* | — | 2·88*F* Right |
| D | 1; 2, 9; 3, 8; 4, 7 | 2·88*F* | 1·00*F* | 1·88*F* Right |
| E | 1; 2, 9; 3, 8; 4, 7; 5, 6 | 2·88*F* | 2·88*F* | 0 — |
| F | 2, 9; 3, 8; 4, 7; 5, 6 | 1·88*F* | 2·88*F* | 1·00*F* Left |
| G | 3, 8; 4, 7; 5, 6 | 0·35*F* | 2·88*F* | 2·53*F* Left |
| H | 4, 7; 5, 6 | — | 2·88*F* | 2·88*F* Left |
| J | 5, 6 | — | 1·88*F* | 1·88*F* Left |
| K | — | — | — | — — |

When fibrils contract in the above sequence, the fibrillar contractions are balanced about the line *WV*, but tend to distort the cilium about the line *XY*. This assumes that all the contractile fibrils lie equidistant from the centre of the cilium and are spaced at equal intervals around the cilium, and that each is capable of exerting the same contractile force. It follows that, when fibrils contract, the bending moment acting to distort the cilium will depend on the distance of the contracting fibrils from the axis *XY*. The relative distances of fibrils from the axis (as calculated) are shown in Text-fig. 3 B, where the largest distance (fibril 1) is taken as 1. In the sequence of contraction stages shown in Table 1, each fibril is assumed to exert a force *F* at distances from the axis shown in Text-fig. 3 B, and to contract in the sequence described above. These data show that the cilium bends first to the right (all diagrams, etc., are orientated as Text-fig. 3 A), the greatest curvature being in stage C. By stage E all the fibrils are contracted and the bending forces on the two sides exactly balance, so that the cilium is straight. In the following stages the fibrils relax and the cilium bends to the left, with a maximum bending moment in stage H

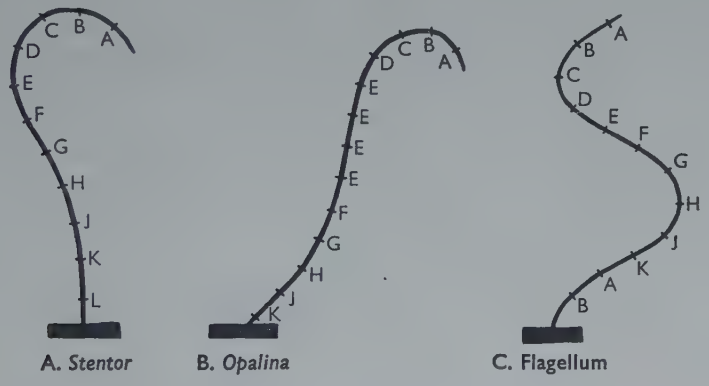
and complete relaxation by stage K. It is also apparent from the data in Table 1 that if the sequence of contraction stages is reversed, so that the contraction starts with fibrils 5 and 6 instead of with the single fibril 1, then the bending forces follow a similar pattern, though not an identical one.

It seems reasonable to suppose that successive excitations are separated by equal time intervals and that each fibril remains contracted for about the same length of time, so that the fibrils relax in the same order as they contracted. Two variables of the timing of the process are obvious. First, the interval between the excitation of successive fibrils may vary and affect the intervals between all the stages. Secondly, the time for which the fibrils remain contracted may vary and this will determine how long stage E persists. The form of beat should therefore depend on how these two variables are related to the frequency of beat and the rate of propagation of the contraction wave along the fibrils.


Text-fig.. 4. The change of bending moments at different stages of contraction. Graph A in crosses and graph B in dots. See explanation in text.

This picture of the contraction process assumes that the fibrils contract and relax instantaneously and remain at a constant tension for five of the contraction stages in Table 1, so that the sequence of effective bending moments can be shown graphically as in Text-fig. 4A. It is unlikely that the contraction process follows this pattern for a number of reasons (e.g. viscous-elastic forces will not allow instantaneous change in length) and Gray (1955) used a sequence of fibrillar tensions following a sine curve in working out a system analogous to that of a sperm tail. On present-day evidence, the contraction process in cilia can be expected to have properties similar to that in muscle, which has been shown to contract and relax quickly, but not instantaneously, with the relaxation normally taking longer than the contraction. Further, it is known that muscles from different sources have different time constants of contraction and relaxation. Gasser & Hill (1924) figure the 'supposed internal mechanical change' of tension in a contracting muscle; the muscle relaxation took about five times as long as the contraction, although the

muscle was half-relaxed in about $1\frac{1}{2}$ times the contraction time. This relative timing seems common in muscle contractions and may be applicable to contractions in the ciliary fibrils. Text-fig. 4 B shows a modification of Text-fig. 4 A in which each fibril is assumed to take two of the contraction stages to reach full contraction and ten stages for relaxation. The relative size of the tension at each stage was calculated from the curve of Gasser & Hill, assuming that the maximum force F was exerted at the second stage of contraction in each fibril. From Text-fig. 4 B it appears that the forces acting on the cilium at various stages will cause it to contract quickly to one side, straighten out as this bend travels up the cilium and all the fibrils contract, and then give a longer but smaller contraction to the opposite side before finally straightening out again when all the fibrils are relaxed. This asymmetry of the contraction process will further modify the form of beat of the cilium and the beat will depend very much on the time constants of the fibrillar contraction process.




Text-fig. 5. Diagrams of cilia and a flagellum at particular stages of their beat to show the application of the theoretical sequence of contraction stages described in the text.

### (*b*) *Explanation of observed ciliary contractions*

The sequence of contraction forces indicated in Text-fig. 4 B would be expected to produce ciliary bending similar to that shown in Text-fig. 5 A, where a short strong contraction to the right (which was responsible for the effective stroke when it started from the base of the cilium) is closely followed by a longer and weaker contraction to the left. The beat of a *Stentor* cilium is therefore explicable along these theoretical lines. Since the whole ciliary beat takes about 36 msec. at 18·5° C., it appears from the form of beat that each contraction stage in Text-fig. 5 A takes nearly 2 msec. This allows complete contraction of a short section of the fibril to take about 4 msec., which is comparable with contraction times found in some fast muscles; it also gives an interval between successive fibrillar excitations of about 2 msec., a time comparable with synaptic delay in the transmission of nerve impulses.

The ciliary beat of *Opalina* is much slower, but has similar features. The whole beat takes on average about 400 msec., so that each fibrillar contraction is very much drawn out. Text-fig. 5 B shows that this beat can be explained by a sequence

of stages similar to that in *Stentor*. After the initial bend of the cilium to the right in the first five stages, the cilium remains straight for about one-quarter of the cilium length, while the fibrillar contractions balance (figured as an extension of stage E), and then there is a long gradual bend to the left as the fibrils relax. It appears that the fibrils remain contracted for some time before relaxing gradually. In this case each stage of contraction and excitation must take something like 10 msec. The ciliary beat of *Opalina* is complicated because the single cilia are fairly flexible when no fibrils are contracted, and while any part of the cilium is in this flexible state it appears to trail in the water as the animal (or that part of the cilium below it) moves, for whatever the direction of movement of the animal, the uncontracted cilia all lie in the direction of the water current over the surface of the animal. *Opalina* cilia show a similar form of beat when beating in any direction, so it appears that there is no set path or system within the ciliary bases of this animal determining the sequence of excitations of the fibrils. If the contraction is visualized as starting in any one of the nine outer fibrils, then there should be nine definite planes of beat. The patterns shown by the metachronal waves are more variable than can be accounted for by this idea, and it seems more likely that the contraction may start with any single fibril or any pair of adjacent fibrils.

Flagellar beating should be explicable by a similar sequence of contractions. Text-fig. 5C shows that the symmetrical beating of a flagellum is related more closely to the sequence of bending forces shown in Text-fig. 4A than those in Text-fig. 4B. Two possible reasons can be put forward for this; first, that the time constants of the fibrillar contraction in the flagellum are such that the relaxation takes approximately the same time as the contraction, and secondly, that the timing of the excitation is such that successive bends on the two sides alternate regularly at equal intervals, whereas in the ciliary beat the interval between bends to left and right is far from equal to the interval between bends to right and left. Gray (1955) found that in sea-urchin sperm tails the beat was seldom symmetrical, for usually the tail bent more to one side than the other. Gray's diagrams of asymmetrical beating show similarities between these sperm tails and cilia, not only because the bend on one side was stronger than that on the other, but also because the weaker bend lasts longer than the stronger one. Both the system of excitation controlling the regular alternation of bending on the two sides and the asymmetry of the fibrillar contraction-relaxation cycle may be involved here, and one is tempted to say that either these two are linked in some way, or more likely that the effects of the former normally mask any minor changes of the latter in a symmetrically beating flagellum. Gray found various degrees of asymmetry in the echinoderm sperm tails, and it seems reasonable that we should regard these as intermediate in form of beat between the 'typical' flagellar beat of the symmetrically beating sperm tail and the type of ciliary beat described for *Stentor*. The main difficulty in interpretation of the sperm-tail diagrams in this respect is that the sperm head moves when the tail contracts. The similarity between the contraction process in these sperm tails and the *Stentor* cilium is emphasized by the almost identical values found for the rate of propagation of the contraction wave in these two organelles.

No mention has been made of the function of the two central fibrils. In *Opalina*, at least, their orientation cannot be related to the direction of ciliary beat, although it appears from the work of Fawcett & Porter (1954) and Bradfield (1955) that they are usually arranged as shown in Text-fig. 3 A. The most promising suggestion on present evidence is that they, together with the internal fluid pressure, are responsible for maintaining some rigidity in the cilium.

It is most significant that the ciliary beat has been found to be a single continuous process in which the functional effective and recovery phases overlap, both being produced by the same flexure as it passes up the cilium, rather than a double process composed of distinct effective and recovery strokes.

## SUMMARY

1. The patterns of beat in cilia of *Stentor* and *Opalina* are described. A fibrillar contraction mechanism is suggested to explain the beating of these cilia and of flagella. The diverse forms of beat are thought to result from differences in the relative timing of parts of the contraction process in different organelles.

2. In both types of ciliary beat the effective and recovery phases are in progress simultaneously and are inseparable parts of one continuous contraction process.

3. The average rate of propagation of the contraction wave in *Stentor* cilia was 844 $\mu$/sec., which is comparable with the rate found in echinoderm sperm tails. In *Opalina* cilia the rate was much slower at between 20 and 100 $\mu$/sec.

4. The peristomial cilia of *Stentor* always beat in the same direction, but *Opalina* cilia may show co-ordinated beat in any direction, and the form of beat appears the same whatever the direction of beat.

It is a pleasure to thank Prof. J. E. Harris, F.R.S., for helpful discussions and criticism.

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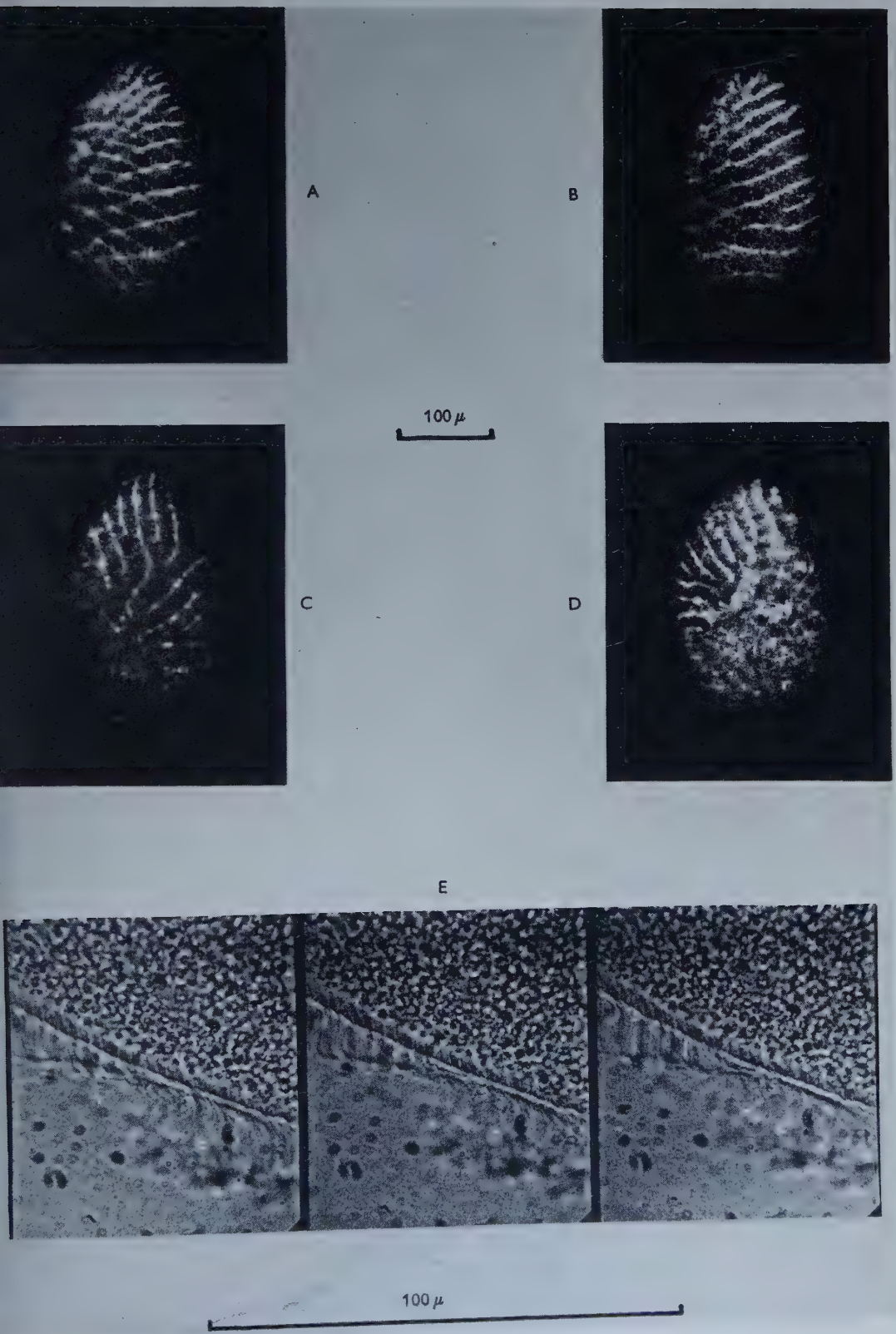
## EXPLANATION OF PLATE

A–D. Photographs of *Opalina* under dark-ground illumination to show metachronal waves. In (A) the waves on both dorsal and ventral surfaces can be seen. In normal swimming (B) the waves pass backwards over the dorsal surface, but when the animal turns to the left (C) the waves travel from left to right, and the waves pass from right to left when the animal turns to the right (D).

E. A series of three successive ciné photographs of cilia of *Opalina* during the passage of a metachronal wave.




SLEIGH—THE FORM OF BEAT IN CILIA OF *STENTOR* AND *OPALINA*

# THE MECHANISM OF GILL VENTILATION IN THE DOGFISH AND SKATE

By G. M. HUGHES

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(*Received* 10 *September* 1959)

## INTRODUCTION

The general nature of the respiratory movements of cartilaginous fishes is well known, especially since the opening and closing of the valves which cover the individual gill slits are very obvious in the dogfish and sharks as are the 'winking' movements of the spiracular valve in skates and rays. But despite descriptions of the mechanism of ventilation, particularly in the dogfish, in many text-books of zoology most of these descriptions are inadequate in detail. Darbishire (1907) gave a good account of the respiratory movements in the dogfish (*Scyllium canicula*), paying special attention to the direction of the water current in the spiracle not only in the dogfish but also in the angel fish and skate. In doing this he noted that carmine particles which entered the spiracle escaped chiefly through the anterior two or three slits, whereas water entering the mouth was ejected from the last three gill slits. No adequate explanation of this difference in flow has been suggested. Darbishire also drew attention to the importance of the spiracle for the entry of water in the skate and to the 'spouting' which occurred through this opening when the direction of the current was violently reversed. This behaviour was similarly described by Rand (1907) who noted that during 'spouting' the gill openings must be closed by muscular action. As Mines (1913) stressed, this phenomenon seems to be a regular part of the breathing behaviour of flattened cartilaginous fishes, although it may also be induced by tactile or chemical stimulation near the spiracles. Baglioni (1907) drew attention to the differences in ventilation mechanism of pelagic and benthic selachians and incorporated them in a system of classification similar to that for bony fishes. As with the latter group he considered that the respiratory current was largely produced by the action of a buccal force pump. Woskoboinikoff (1932), on the other hand, emphasized the importance of the pumping action of the gill pouches themselves, and maintained that this was the most primitive mechanism, as it is the sole mechanism in the Agnatha. Balabai (1939), however, from measurements of the maximum and minimum pressures in the oro-branchial and para-branchial cavities, suggested that as in teleost fishes (Woskoboinikoff & Balabai, 1936, 1937; Hughes & Shelton, 1957, 1958), water passes across the gills as a result of the combined action of a force pump in front and a suction pump behind the gill lamellae. Teichmann (1959), in a comparative study published since the present work was carried out, has given evidence concerning the relative importance of these

two pumps in *Scyliorhinus*, *Mustelus* and *Torpedo*. In the present work attention has been paid to the time-course of the ventilation movements and associated pressure changes in the different cavities of the respiratory apparatus in the dogfish and skate.

## MATERIALS AND METHODS

The dogfish used in this work was *Scyliorhinus* (*Scyllium*) *canicula* (L.) and the fish referred to as 'the skate' was in fact the thornback ray, *Raia clavata* L. Specimens had been freshly caught and kept in the sea-water circulation at the Plymouth Laboratory of the Marine Biological Association, whose aquarium was of great value for watching the normal respiratory movements of these animals. The size of the animals varied, but usually the dogfish weighed about 600 g. and the skates about 400 g. All experiments were carried out under light anaesthesia. First of all the animals were deeply anaesthetized before they were fixed in the experimental tank, where they were allowed to recover to a state of light anaesthesia. In no case was the depth of anaesthesia sufficient to stop the breathing movements completely. The anaesthetic used in the first series of experiments was urethane, but it was found in later experiments that MS. 222 (Gilbert & Wood, 1957) gave much more consistent results, the animals showing a constant pattern of breathing for several hours which was indistinguishable from that of a resting unanaesthetized animal. The concentration used varied according to the size of the animal, but was usually about 1 in 50,000. The dogfish were held in a larger version of the clamp used in studies of freshwater teleosts (Hughes & Shelton, 1958; Shelton, 1959), but in many cases it was found that the body need be clamped only very lightly. Holding the skate was not so easy, but an adequate method was found by lightly bandaging it to a brick which was placed in the experimental tank. Most experiments were done with the skate's ventral side uppermost in order to make it possible to insert the pressure-recording needles into the gill slits. As the spiracle is on the dorsal side it was difficult to obtain simultaneous recordings of the spiracular and gill slit pressures, but this could be done if the fish was held so that the spiracle did not lie over the brick. The experimental tank of about 25 l. capacity was filled with fresh sea water containing the anaesthetic and was kept constantly aerated throughout the experiments. The temperature of the water was 10–12° C.

The pressure-recording apparatus was similar to that already described (Hughes, 1958; Hughes & Shelton, 1958). Two Hansen condenser manometers, one utilizing a modified high-frequency circuit (Machin, 1958), were used in the main part of the present study. An Ediswan four-channel pen recorder made it possible to record simultaneously the pressures in two places together with the corresponding movements. As the frequency response of the pen recorder was flat up to 90 cyc./sec., which was also true of the electric manometers, the wave-forms were faithfully reproduced. Movements of the mouth and branchial apparatus were recorded by means of RCA 5374 mechano-transducer valves. Simultaneously with the pen recordings it was possible to record any two of the four channels on a Cossor 1049 double-beam oscilloscope, and these oscillograms were used for more detailed analyses of the wave-forms especially when comparing the pressures at

particular instants, as this avoided errors due to the alignment of the pens and the curvatures of their recording arcs. In the initial experiments on the dogfish some films were taken simultaneously with pressure and transducer records, and these results proved of value for comparison with the results obtained later using the four-channel recording apparatus. These films, together with results obtained on freshwater fishes, have confirmed that the use of a long light arm attached to the anode of the RCA 5374 gives recordings of the respiratory movements which are essentially the same as those obtained by the more laborious analysis of ciné films. Films were also taken of unanaesthetized dogfish and skates, and also some of animals which though under light anaesthesia were not restrained in any way. Of particular value in ascertaining the time relations of the movements of the spiracular valve in the skate were films taken of young animals about 5 in. in fin span. With these it was possible to photograph both dorsal and ventral surfaces simultaneously by making use of a suitably placed mirror. Indian ink or milk adjusted to the same specific gravity as the water was used to follow the course of the respiratory stream. A Zeiss Movikon 16 mm. camera was used at speeds of 16–32 frames/sec., and lighting provided by a pair of photofloods.

## RESULTS

### I. *The respiratory apparatus in selachians*

The most characteristic feature of the gill apparatus in cartilaginous fishes, contrasting with that of bony fishes, is that the respiratory current passes out by a number of openings on each side. The presence of these separate gill slits externally is due to the well-developed septa which separate the two rows of gill filaments attached to a given branchial arch. The number of gill slits varies from five to eight, but in the species used in the present investigation there are five pairs, which is the number most generally found throughout the group. The gills themselves are characterized by the formation of gill pouches which communicate with the pharyngeal cavity by relatively large internal gill openings. Functionally the gill pouches together with the bucco-pharyngeal cavity form a single cavity which will be referred to as the *oro-branchial cavity*. The gill pouches may be compressed by the action of some of the intrinsic musculature of the visceral arches and their expansion is largely due to the elasticity of this skeleton but may be aided by some antagonistic muscles. No attempt has been made in the present study to determine the precise roles of the many different groups of muscles involved in the respiratory movements except in so far as these can be judged from the records and their known morphological disposition. When the water current has passed over the gill filaments it collects into cavities which run dorso-ventrally beneath the lateral walls of the branchial region in the dogfish and mainly horizontally beneath the ventral surface in the skate. These cavities were named by Woskoboinikoff (1932) the *parabranchial cavities* and his nomenclature will be adopted here. In the dogfish and skate they are only open to the exterior for a small part of their length but in other selachians, e.g. *Cetorhinus*, the basking shark, their openings are relatively much longer.

Pressure measurements were readily made from these cavities and provide useful information about the mechanisms by which the flow of the respiratory current across the gills is maintained. Collectively they are analogous to the opercular cavities in the teleost fish. The buccal cavity of the latter corresponds to the oro-branchial cavity. A further difference from the teleost fish is the persistence of the second visceral slit as a spiracle through which water may enter or leave the oro-branchial cavity. This opening is guarded by a valve on the anterior side of the cleft which can be actively closed by the action of the first dorso-constrictor muscle.

## II. *The Dogfish*

It must be emphasized that the results described below all relate to the respiratory movements of animals which are stationary relative to the water. Since these experiments were carried out it has been possible to observe other selachian fishes at the Marineland Aquarium, California. In one of these, the leopard shark (*Triakis semifasciata*), it is quite clear that during normal swimming the mouth is held wide open and the gill flaps are also open, water passing in a continuous stream over the gills. As the speed of swimming falls, the fish makes occasional pumping movements which become more frequent when it rests on the bottom. As far as could be judged from the films taken, these movements are identical with those described below.

### (*a*) *The movements*

When observed at rest or swimming slowly in an aquarium a most obvious feature of the breathing movements is the rhythmical expansion and contraction of the whole branchial region, and the regular opening and closing of the mouth and of the small flaps which cover the five pairs of gill slits during the expansion phase. These movements occur every 1–2 sec. and have components in all three dimensions. The floor of the oro-branchial cavity rises and falls rhythmically as the mouth closes and opens. The walls of the gill region are compressed laterally and also appear to move in a posterior direction as the water is expelled from the gill slits. The presence of these complex movements makes them difficult to record and to represent adequately in even a diagrammatic way. However, a combination of the analysis of films taken in side and dorsal views and of the movement records gives a fairly consistent picture. The use of milk adjusted to the specific gravity of sea water pipetted near the mouth and spiracular openings made it possible to confirm the observations of Darbishire (1907) on the direction of the current. It is quite clear that this tends to be unilateral, for water entering a spiracle only leaves through the three anterior gill slits on the same side. Normally there is very little reflux through the spiracle although this is strong during the occasional 'spouts'. The valve guarding this opening is an active one and it must be efficient otherwise it would short-circuit the main flow through the gills. There appeared to be a certain amount of reflux through the mouth as it closed and the oro-branchial cavity decreased in volume. The passive maxillary and mandibular valves are not well developed in *Scyliorhinus* and there must be some loss of water in a stationary fish. Water entering

the mouth escapes through the last three gill slits, and when milk is pipetted into one side of the mouth it issues from the gill slits on the same side.

The decrease in volume of the oro-branchial and parabranchial cavities takes place comparatively rapidly during about one-quarter of a cycle and is succeeded by a slower phase of expansion. The latter is fairly rapid to start with but becomes progressively slower. During this expansion phase the extensions of the gill septa which form valves over the gill slits are seen to be 'crinkled' as if drawn by suction from within. A more or less distinct pause occurs following the expansion phase, during which the gill flaps become slightly free from the body wall, the onset of the next expiratory phase being marked by the synchronous and rapid opening of these valves. The dorso-ventral movement of the buccal cavity always precedes the lateral

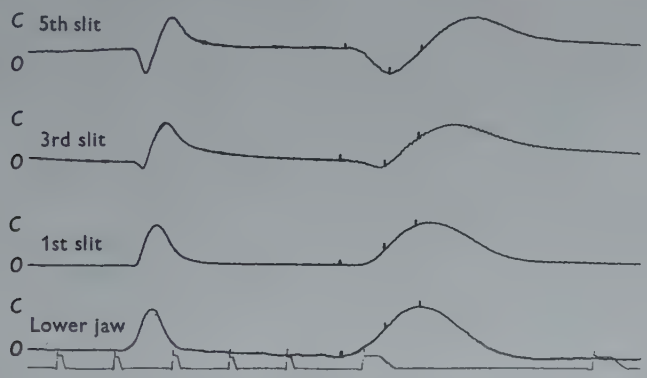


Fig. 1. *Scyliorhinus*. Mechano-transducer recordings of movements of the lower jaw and branchial region during two breathing cycles. The recording levers were placed just in front of the corresponding gill slit. Closing of the mouth and adduction of the branchial arches is upwards in all cases as is indicated by '*C*'. Three identical instants are marked on each of the tracings. Time marker = 1 sec. *Adduction* is used to describe the movement analogous to adduction of the operculum in teleost fish, i.e. movement towards the mid-line of the body as seen from the dorsal side.

expansion of the gill region. This has been recorded many times by means of the transducers. A most convenient point for recording this movement with little interference to the gill movement was just in front of the first gill slit, where the head of the dogfish is widest. On other occasions movements of this region were recorded more posteriorly from positions between and just above a pair of gill slits. The latter records repeatedly indicate that when recording close to a valve a brief abduction precedes adduction of the branchial arch. Furthermore, the relative size of this abduction increases in recordings taken from the more posterior slits. This is most clearly shown in Fig. 1, where the four-channel recorder was used with transducers on all channels. These records show that the first movement to be recorded, whether it be an abduction or adduction, is simultaneous throughout the whole gill region but that if one considers the adductor movements alone then these spread from in front backwards. This is also true of the expansion movements of the branchial region, i.e. the first gill pouch begins to expand before the second, etc. Another difference to be noticed in these records and which is connected with

the preceding observations is that the rate of the adductor movements is more rapid in the more anterior arches. The same is also true of the succeeding more rapid phase of expansion.

The complete interpretation of these recordings must be deferred until after the pressure changes in the parabranchial cavities have been described.

*(b) Pressure relationships of the respiratory cavities*

The time-course of pressure changes in different parts of the respiratory system is very similar in its general form (Figs. 2 and 5). It includes times when the cavities are positive or negative, in addition to periods when the pressure is the same as that

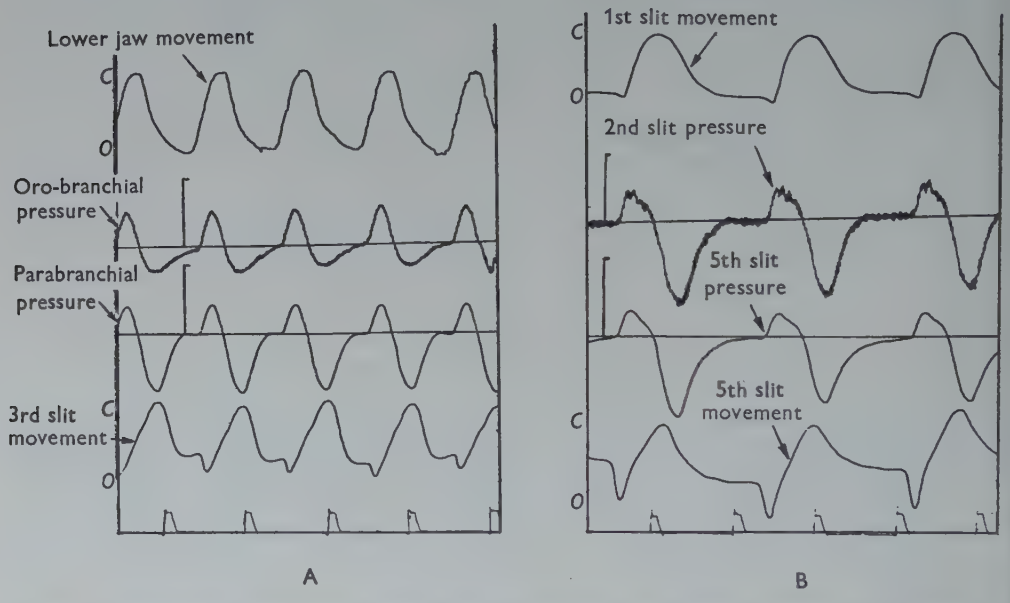


Fig. 2. *Scyliorhinus*. Four-channel recordings of movement and pressure changes in (A) the oro-branchial cavity and parabranchial cavity of the 3rd gill slit. (B) Parabranchial cavities of the ipsilateral 2nd and 5th gill slits. As in all other recordings of this type, the pressure recordings are the two middle tracings and the corresponding movement record is next to it. Calibration pressure is +1·0 cm. water with respect to the zero pressure. Time marker = 1 sec.

of the outside medium, which will be referred to as zero pressure. The relative size and duration of these different parts of the cycle vary according to the position of the cavity. There are also variations between different individuals, but the present account is based upon the pattern found in about 70 % of the experimental animals. Pressures recorded from the oro-branchial cavity with a needle inserted into the mouth or through the spiracle both showed that the maximum positive pressure attained (0·5–1·5 cm. water) was greater than the maximum negative pressure (0·2–0·5 cm.). The opposite was true of the pressures recorded from any of the parabranchial cavities. In the latter the negative part of the curve is predominant and reaches a maximum of 1–2 cm., whereas the maximum positive pressure attained does not

usually exceed 1 cm. Some differences were observed in the pressure curves recorded from different gill slits. For instance, Fig. 2 B shows simultaneous recordings from the first and fifth gill slits on the same side. It can be seen that the general wave-forms are similar, but closer inspection reveals that while the positive phases are about equal in magnitude, the maximum negative pressure in the more anterior slit is greater. Furthermore, it is noticeable that the rate of fall of this pressure is much more rapid than in the case of the pressure recorded from the fifth parabranchial space. The oscilloscope records (Fig. 3 B) confirm these features, and also show a slight phase shift between the two curves, since pressure changes in the more anterior slits precede the corresponding changes in the posterior slits by a short but perceptible time.

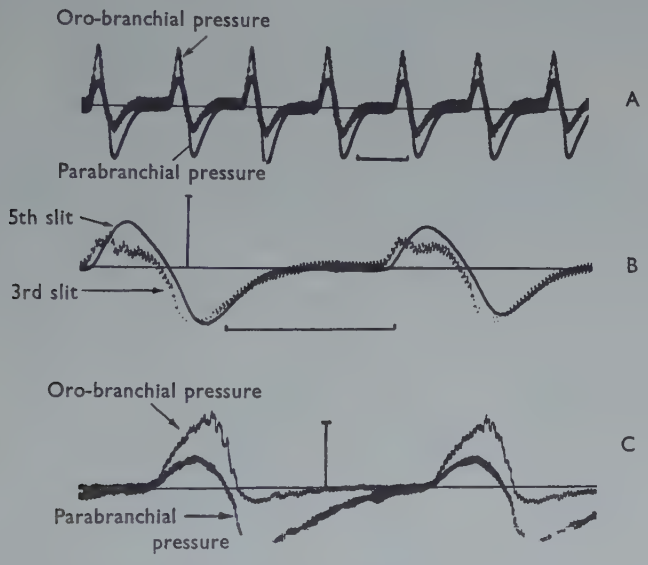


Fig. 3. Oscilloscope records of pressure changes in the respiratory cavities of *Scyliorhinus* (A and B), *Raia* (C). A. Oro-branchial and 5th parabranchial cavities. B. 2nd and 5th ipsilateral parabranchial cavities. C. Oro-branchial and 3rd parabranchial cavity. Calibration = +1.0 cm. water. Time = 1 sec.

This phase difference between the wave-forms is even more obvious when simultaneous recordings from the oro-branchial cavity and one of the parabranchial cavities are compared (Fig. 3 A). The oro-branchial cavity becomes positive a little sooner than the parabranchial cavities and likewise it usually becomes negative before the parabranchial cavities. This latter feature means that, just as in the teleost fishes, there is a brief phase (4) during a cycle when the two curves cross and the direction of the differential pressure reverses, thus tending to produce a reversal in the direction of the respiratory current. The differential pressure curve (Fig. 4) shows that a gradient exists from the oro-branchial to parabranchial cavities except during this transition when the oro-branchial cavity begins to increase in volume.

The pressure curves make it quite clear, therefore, that the flow of water is

maintained by the action of two pumps as in teleost fishes. One of these (the *force* or *pressure pump*) depends on a greater positive pressure from the oro-branchial to parabranchial cavities and the other (the *suction pump*) involves a greater negative pressure in the latter cavities.

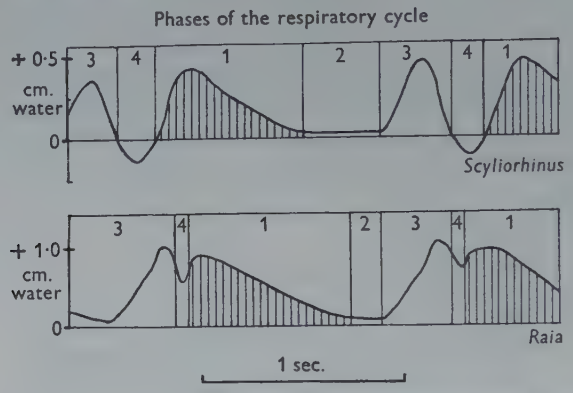


Fig. 4. Curves of differential pressure between the oro-branchial and third parabranchial cavities of *Scyliorhinus* and *Raia*. A positive differential pressure indicates that the oro-branchial pressure is greater than the parabranchial pressure. The numbering of the phases of the respiratory cycle is the same as in Hughes & Shelton (1959). Phase 1, when the suction pump predominates, has been shaded.

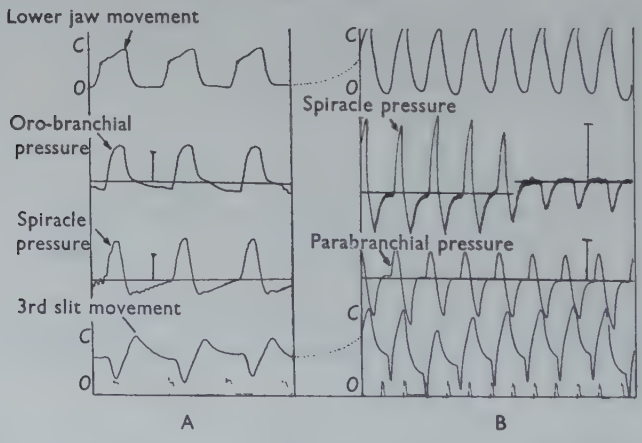


Fig. 5. *Scyliorhinus*. Pressure changes recorded through the spiracle together with those in (A) the oro-branchial cavity (needle inserted through mouth) and (B) the 3rd parabranchial cavity. In B the pressure is shown inside the spiracular valve and then following withdrawal of the needle to just outside it. Movement records in both A and B are from the lower jaw and in front of the 3rd gill slit. Calibration = +1·0 cm. water. Time marker = 1 sec.

*Functioning of the spiracle.* As mentioned above, the general form of the pressure curve recorded when the needle was placed within the spiracle was similar to that recorded from the oro-branchial cavity by needles inserted through the mouth (Fig. 5). The two pressure curves are similar in that the positive part is predominant in both, but they differ in detail. This is seen in the rate of fall of the positive pressure, which is much more rapid and occurs a little sooner when recordings are made from

the spiracle than from the mouth or any of the gill slits. As the spiracular pressure returns to zero that of the oro-branchial cavity is more negative. Thus the pressure gradient drawing water into the oro-branchial cavity is greatest first of all via the spiracle and later via the mouth. This would suggest that the first water to enter this cavity will be through the spiracle, and it is reasonable to suppose that it will enter the anterior gill pouches. A further factor contributing to this must be that water entering the spiracle will tend to take up a more lateral position in the flow through the oro-branchial cavity, which is likely to be laminar on account of its relatively low velocity. When the needle was gradually withdrawn from the spiracle a point was reached where the positive phase disappeared. In this position the pressure was being recorded just outside the spiracular valve where only a negative pressure is present. If the needle was carefully manœuvred until it was directly opposite the valve it was possible to obtain a small positive deflexion as the valve closed.

(*c*) *The relationship between pressure changes and movements*

In general this is fairly clear and what might be expected. Thus as the mouth closes the pressure in the oro-branchial cavity rises and remains positive until the oro-branchial cavity begins to expand again. It then becomes negative, water is drawn in through the mouth, and the pressure rises to be the same as that outside. The pressure recorded from the spiracle has similar relationships, but it usually becomes positive a little later than that recorded from the mouth. The spiracular pressure becomes positive when the spiracular valve closes. The form of the curves recorded from the parabranchial cavities and the corresponding movement records are not always so easily interpreted. Movement recordings from the first slit can be related to the pressure changes in the parabranchial cavities quite easily as during the adduction movements of this arch the pressure rises and during abduction it falls, becoming negative. Movement recordings from the more posterior slits, however, give the impression that the arch is maximally adducted as the pressure in the corresponding parabranchial cavity becomes negative and similarly the commencement of the positive phase seems to coincide with the brief abduction which precedes the main adductor movement.

These observations become easier to interpret if the movement recordings from near the gill slits are considered in two components. One of these is due to the activity of the branchial musculature of the arch concerned, and the second is produced indirectly as the result of pressure changes to which the pouch is subject by the action of the force pump. Thus the initial abduction is entirely due to the passive opening of the valves as water is forced out at the beginning of the decrease in volume of the oro-branchial cavity. In confirmation of this it can be shown that this abductor movement decreases and may disappear if the recording lever is moved away from the valve. In some experiments when the pressure needle was inserted more deeply into a gill pouch an extra positive deflexion was found which preceded the normal positive wave of the parabranchial cavity. This extra deflexion was synchronous with the positive pressure in the oro-branchial cavity and also with abduction of the valve. Correspondingly at the other transitional phase when the oro-branchial

cavity begins to expand and the pressure differential is temporarily reversed, some movement records indicate an extra adduction which is due to the 'sucking in' of the valve. Again, such recordings are only found when the transducer arm is placed just in front of a slit where it is most affected by the valve itself.

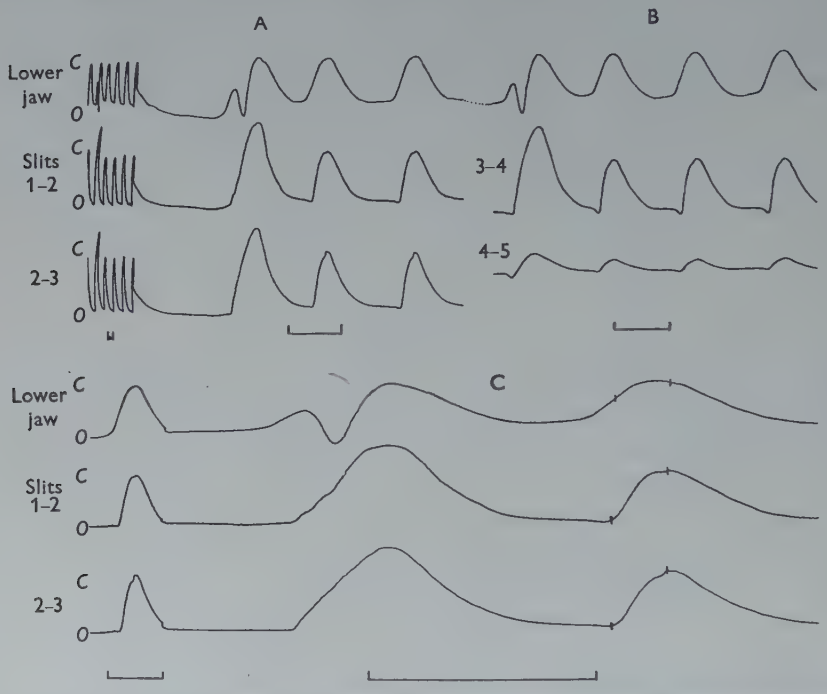


Fig. 6. *Raia.* Mechano-transducer recordings of movements of the lower jaw and across differen pairs of gill slits. Recordings were made at different speeds; time marker = 1 sec. in all cases.

## III. *The Skate*

In a skate resting quietly at the bottom of an aquarium it is apparent that water enters via the two dorsally situated spiracles whose valves open and close rhythmically with a frequency of about 30/min. When the animal is more active and swimming about, this frequency increases and water also enters through the mouth. This was also true under the conditions of the present experiments, and it seems to be normal when the animal is resting with its snout slightly raised (Rand, 1907). This posture can often be observed in the guitarfish (*Rhinobatos productos*) at the Marineland aquarium. When the skate is almost completely buried it seems unlikely that the mouth will be opened. Rand states that the mouth and spiracular valve close together but that opening of the mouth is slightly later than that of the spiracle; but other accounts suggest they are synchronous in all phases. Analyses of both films and transducer records show quite clearly that the mouth precedes the spiracular movement during both the opening and closing phases by about one-tenth of a cycle. Expansion and contraction of the branchial region are also delayed

with respect to the mouth movements (Fig. 6). Movements of the individual gill pouches are more nearly synchronous than is the case in the dogfish, but a slight delay can be seen between the movements of the first and last gill arch. This is true for both the expansion and compression phases. The spiracle is definitely closed by an active valve, and although the flaps which cover the gill slits appear to be passive, there is evidence for a mechanism which actively closes the parabranchial cavities to the outside. The greater synchrony of action of the gill pouches is to be expected from the structure of both skeletal and muscular systems, and is due in part to the fusion of the basibranchial cartilages into a copula.

The respiratory current leaves through the five pairs of gill slits but during more intense breathing the use of dyes in the water shows a very slight reflux through both the oral and spiracular openings. Under these conditions, which were sometimes found towards the end of an experiment, another very characteristic feature of the respiratory rhythm is the periodic 'spouting' of water through the spiracle which was observed by Darbishire (1907) and Rand (1907). This activity may be due to a lack of oxygen or to mechanical stimulation of sense organs in the spiracle by mucus or other foreign bodies in the water but it is also a part of the normal respiratory cycle (Mines, 1913). The current entering via the right spiracle or right side of the mouth usually leaves by the gill slits on the same side. There is a tendency for water entering by the spiracles to leave by the anterior gill slits, and for that which enters by the mouth to emerge through the posterior slits, but this is not so marked as in the dogfish.

The pressures recorded from the oro-branchial and parabranchial spaces show that the flow of the respiratory current is normally maintained by the action of both force and suction pumps. The oro-branchial pressure curve has a more positive phase than that of the parabranchial curve, which has the greater negative phase (Figs. 3 C, 7). The positive phase of both pressure curves is associated with a decrease in volume of the cavity and the negative pressure likewise associated with an increase in volume. The latter is about two-thirds of the respiratory cycle and the former one-third of the cycle. As in the typical teleost fish, these two major phases are again separated by phases of transition, but here the two curves come very close together during both transitions. In contrast to the dogfish and most teleost fishes, there does not seem to be any reversal of the pressure gradient which usually occurs as the suction pump takes over from the pressure pump. In fact the opposite is the case here, for the parabranchial pressure falls before that in the oro-branchial cavity. It can also be seen that the two pressures follow the same course during the other transitional phase (2), and in some instances there is a suggestion of a slight reversal. The result of the interaction of these two pumps in maintaining the flow across the gill is shown by the differential pressure curve (Fig. 4). Recordings from any of the five slits seem to be the same except during a 'spout' when the first slit has a longer and more positive pressure than the more posterior slits (Fig. 8 A). During 'spouting' the positive pressure in all of the slits is greater than in the buccal cavity and it is due to this that the water current is reversed and forced out through the spiracles and mouth (Fig. 7).

*The action of the spiracle and 'spouting'.* It has not been possible to record the pressures in the spiracles simultaneously with pressures in the oro-branchial or parabranchial cavities. Attempts to insert a needle into the former via the spiracle at the same time as the spiracular pressure was recorded were not successful because irritation of the sensitive spiracular epithelium immediately caused the animal to move. It was possible, however, to record the spiracular pressure on the two sides

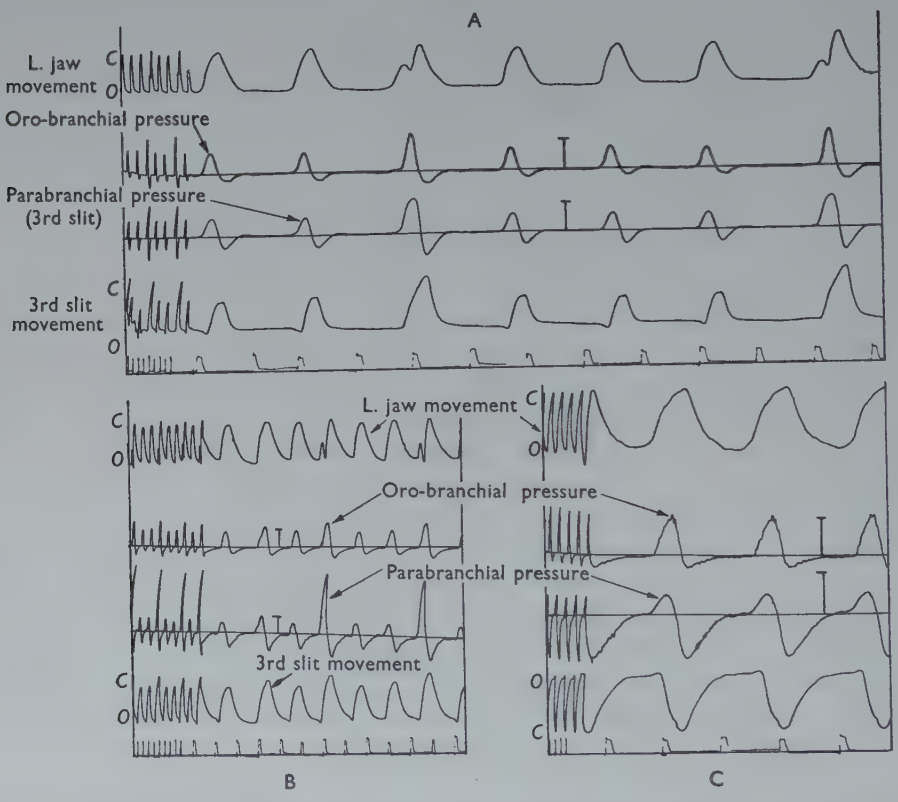


Fig. 7. *Raia.* Four-channel recordings of the movement and pressure changes in the mouth (oro-branchial cavity) and parabranchial cavity of the 3rd gill slit. Recordings are shown from three different experiments and illustrate variations in the frequency and nature of 'spouting'. Record C was taken near the beginning of an experiment when there was scarcely any 'spouting'. Note that the reversal in the pressure gradient during 'spouting' is much greater in B than A. Pressure calibration = +1·0 cm. water. Time markers = 1 sec.

simultaneously with the movements of the spiracular valves. These experiments showed that the positive phase of the spiracular pressure normally coincides with the time of maximum closing of the spiracle (Fig 8 B). As the spiracular valve closes just after the mouth it is probable that these pressure changes in the spiracle occur soon after those recorded in the mouth. The pressure curves from both spiracles were very similar so long as care was taken that the recording needles were in identical positions on the two sides. As in the dogfish, the pressure just outside the valve

has a small negative phase only, whereas once the needle has passed the valve a positive phase becomes apparent. During 'spouting' the spiracular valves only partially close but maintain the normal rhythm. Several times an asymmetry in the action of the two spiracles was observed. Rand (1907) also noticed such unilateral 'spouting'. In one of the spiracles the positive phase was increased, whereas in the other it was less than the normal pressure (Fig. 8B). This decreased pressure still

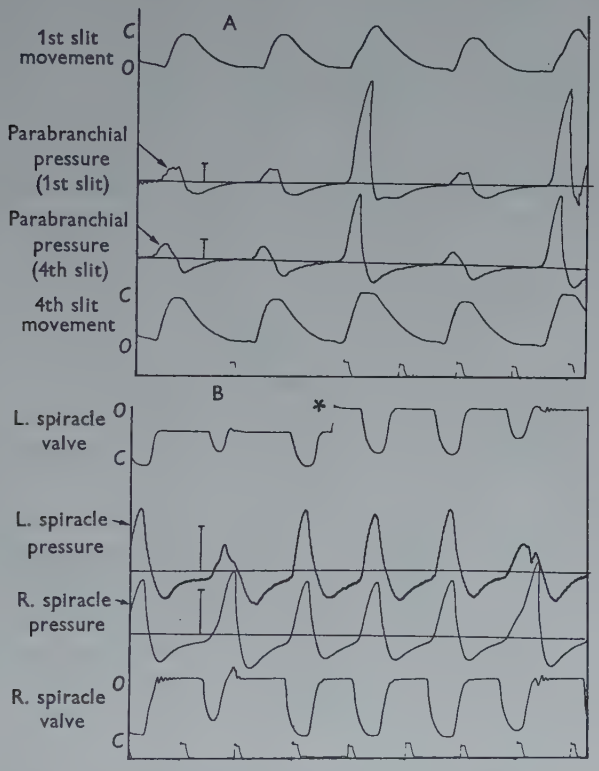


Fig. 8. *Raia.* A. Pressure and movement records from the 1st and 4th gill slits on the same side. The pressures are recorded in the parabranchial cavities and the transducer levers are in front of the corresponding gill slit. B. Recordings of the pressure changes in the two spiracular openings internal to the spiracular valves whose movements are recorded on the upper and lower traces. This specimen was showing 'asymmetric spouting', a greater volume of water being ejected through the right spiracle. In both A and B, recordings during two 'spouts' are shown. Calibration = +1·0 cm. water. Time marker = 1 sec. * Artefact lever slipped.

had fundamentally the same time-course as in the normal rhythm and reached a maximum when the spiracular valve was maximally closed, whereas the side with the increased positive pressure showed a delayed maximum which now coincided with the position of maximum opening of the spiracular valve. Direct observation of the water stream showed that during the normal respiratory cycle there was a slight and equal reflux through both spiracular openings as the oro-branchial and parabranchial cavities decreased in volume, but during the 'spouting' cycle the

greatest reflux occurred through the spiracle with the increased positive pressure. Furthermore, water was leaving when the valve was maximally opened and not when it was nearly shut as was normally the case. During these 'spouting' actions the positive pressure in both the oro-branchial and parabranchial cavities was increased above normal but, as mentioned above, that of the latter became the greater. It preceded and was often more prolonged than the oro-branchial pressure (Fig. 7). The difference in amplitude of these two pressures may be as great as 3–4 times, but in other cases it is only slight. Movement records showed that during this phase the gill pouches contracted a little earlier than normal but maintained the antero-posterior order; but during the succeeding phase they expand simultaneously. The amplitude of their contraction is also increased during a 'spout', whereas that of the mouth remains unchanged. Closing of the oro-branchial cavity is interrupted by a brief expansion phase which coincides with the maximum positive pressure in this cavity. Its continued decrease in volume reaches a minimum which coincides with maximum closure of the gill pouches; thereafter the whole system expands synchronously as mentioned above.

## DISCUSSION

The present investigations have substantiated the view that the flow of water through the gills of two species of cartilaginous fish is maintained by the action of two pumps which operate on both sides of the gills. The time-course of pressure recordings in these two positions makes this quite clear and gives a good indication of the nature of the flow across them. As with the teleost fish, the flow is probably more or less continuous. The existence of a pause between two successive cycles in *Scyliorhinus* (as was also noted by Satchell (1959) in *Squalus lebruni*) when the pressure throughout the whole system is very close to that of the external medium suggests that any flow during this part of the cycle must be extremely slow. Phase 2 of the differential pressure curve (Fig. 4) indicates this, but it has been pointed out (Hughes & Shelton, 1958) that the effect of possible changes in gill resistance must always be borne in mind when interpreting these curves.

This is true, for instance, when using the pressure curves to decide the relative importance of the two pumps in maintaining the flow across the gill resistance. From evidence at present available, however, the areas beneath phases 1 and 3 of the differential curve certainly give a fair indication of the work done by the suction and force pumps respectively. If the resistance remained constant throughout all phases of the respiratory cycle, it would be possible from these areas to express quantitatively the relative importance of the two pumps in maintaining the flow across the gills. Even allowing for some change in resistance the curves provide good evidence that the suction pump plays an important part in both the species investigated and that its importance is greater in the skate than in the dogfish. This result agrees with that of Teichmann (1959) for *Torpedo*, but is at variance with his conclusion that the force pump does most of the work in *Scyliorhinus*. He noted, however, that in some individuals the suction pump was more important than in the experiment which he quotes. Such individual variations seem to be very

characteristic of dogfish, for Balabai (1939) found quite large variations in the relative magnitude of pressure changes in the two cavities. In some specimens the orobranchial cavity became both more positive and more negative than the parabranchial cavity. Certainly individuals have been found in the present investigations in which the force pump was better developed than that shown in Fig. 4, but in no case is it the only mechanism. Of course if the gill resistance should fall during this phase similar positive pressures will be recorded on either side of the gills and there will be very little differential pressure across them, although the force pump is actively expelling water from the whole system. What matters most, however, is the work done by the pumps in ventilating the gills, and if the resistance of the gills is low it is because a smaller proportion of water is passing between the secondary lamellae. Hence in terms of ml. of oxygen absorbed per unit of work done by a pump, the differential pressure is a good guide to its importance.

Teichmann's method of assessing the relative importance of the two pumps was to measure the maximum height to which they could force water above the surface when one of the two pumps was functionally disturbed. As he points out, the animal can and usually does compensate for these disturbances. Interference with the force pump by placing a tube into the mouth of *Scyliorhinus* decreases the height by about two-thirds, but this does not necessarily mean that in the normal respiratory cycle the suction pump plays a very minor role. This method is suitable to show the ability of the pumps to force water out of the system as a whole. As the pressure curves show, this is not the primary function of the suction pump, however, which is particularly adapted to draw water through the gill system. Furthermore, in addition to the maximum pressure achieved, it is also necessary to take into account the duration of the action of the pumps, which is longer for the suction pump, and for this purpose a study of the time-course of ventilation is essential.

One of the most interesting features of the differential pressure curves is the apparent absence of a reversal in the gradient in recordings from the skate. This has also been found in some bottom-living teleost fishes (Hughes, 1960). The simplest explanation appears to be that the opening from the parabranchial cavities to the outside can be actively closed. This would enable a fall in pressure to occur in these cavities before it does in the oro-branchial cavity and hence would maintain the direction of the pressure gradient during this phase. The suggestion of an active control of these apertures is not new, for in an addendum to his paper Darbishire (1907) mentions that Prof. Herdman was of the opinion that the gill covers in the living dogfish were not passive but active agents in determining the respiratory current. Darbishire came to the conclusion that the anterior part of the gill cover which is supported by the gill rays can be moved actively, but that the portion not supported by the rays is entirely passive. Rand (1907) also drew attention to the need for an active mechanism closing the gill slits in order to ensure a reversal in the flow during 'spouting'. The latter is certainly better developed in the flattened forms and correlates with the nature of their differential pressure curve. The value of such a mechanism to the animal probably lies in the fact that it ensures that no water can enter through the external gill slits, which must occur, however

slightly, in a system entirely dependent on passive valves. The danger of particles of sand damaging the gills if they entered by such a route is of course much greater in fish which lie partially buried on the sea bottom.

The actual details of this mechanism require further investigation but certainly the morphology of the gill septa makes possible an active control of the type suggested by Darbishire. Lighttoller's (1939) description of the different layers of constrictor muscles also fits in with his suggestion. It would appear that the dogfish has the structural basis for such control, but that apart from 'spouting' it is used much more rarely or in a different way during the normal respiratory cycle.

## SUMMARY

1. The respiratory movements of the dogfish, *Scyliorhinus* (*Scyllium*) *canicula* (L.), and the 'skate', *Raia clavata* L. (thornback ray), have been studied by the use of cinematographic and mechanotransducer recording methods. Simultaneous determinations of the time-course of pressure changes in the oro-branchial and parabranchial cavities were also made by means of Hansen condenser manometers.

2. In both species movements of the mouth precede those of the spiracular valve and of the branchial region. Adduction and abduction of the branchial region spreads serially from the first to last gill slit in the dogfish, but movements of the individual gill arches are more nearly synchronous in the skate. Opening of the flap valves formed by extensions of the inter-branchial septa are synchronous in both species.

3. Water entering one side of the mouth leaves by the three posterior gill slits of the same side. Water entering the spiracle leaves through the anterior slits of the same side. This separation of flow is less marked in the skate.

4. The pressure curves recorded in all parts of the system have both positive and negative phases with respect to the external medium. The positive phase, associated with closing of the mouth and spiracle, is larger in the oro-branchial than in the parabranchial cavities and vice versa. The time-course of the pressure changes indicates that the flow across the gills is maintained by the action of a pressure pump in front and a suction pump behind.

5. The suction pump plays a more important role than the pressure pump in the skate and its contribution to the flow across the gills is by no means negligible in the dogfish.

6. The differential pressure curves suggest that the flow across the gills is continuous except in the dogfish for a brief period when the gradient is reversed. The absence of this reversal in the skate suggests that the external gill slit openings are controlled by an active mechanism. This is probably an adaptation to bottom-living habit.

7. All these observations relate to animals which are stationary with respect to the water. During swimming at a reasonable speed leopard sharks (*Triakis semifasciata*) have been observed to make few or no respiratory movements, although they immediately ventilate actively on coming to rest at the bottom of the aquarium.

It is a pleasure to record my thanks to the Director and Staff of the Plymouth Laboratory, both for the facilities and material they provided and their friendly assistance throughout the work. I also wish to thank Dr G. Shelton for his collaboration in the early part of this work, and Dr K. E. Machin for his assistance in designing the electronic apparatus.

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# A COMPARATIVE STUDY OF GILL VENTILATION IN MARINE TELEOSTS

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## INTRODUCTION

Relatively little work has been done on the gill ventilation of British marine teleost fishes since that of McKendrick (1879) who made one of the earliest comparative studies of fish respiratory movements. He was surprised by the variability, not only in the frequency but also in the shape of the wave forms recorded by tambours placed lightly against the operculum of different species of fresh-water and marine fishes. Apart from noting these variations he did not attempt any classification of the different mechanisms. Baglioni (1907) was the first to do this when he divided marine fishes into four main groups according to their habits of life and drew attention to the accompanying variations in their respiratory mechanisms. These variations are largely in the degree of development of the branchiostegal apparatus which co-operates with the operculum. The first group he distinguished consists of pelagic fishes which never rest on the bottom. In these the operculum itself is well developed and the branchiostegal apparatus relatively small. The second group is intermediate between these forms and the third group which includes the true bottom-living fishes. In the second group he included such families as the Cottidae, Gobiidae and Blenniidae and pointed out that their respiratory movements are characterized by the greater role of the branchiostegal apparatus. The importance of this apparatus becomes even greater in the third group, some of which frequently burrow in the mud or sand of the sea floor, e.g. Trachinidae Pleuronectidae. In the fourth group, however, a true branchiostegal apparatus is absent. This group is not a homogenous one and it includes such families as the Muraenidae and Syngnathidae. Willem (1927, 1947) has suggested a similar classification but he recognizes six different groups. The two additional ones are mainly due to his detailed studies of the peculiar mechanisms of the plectognaths. Most recently Bertin (1958) has largely combined these two systems but recognizes a further group to include the interesting adaptations found in forms that live in torrential streams (Hora, 1933).

In a study of the respiratory mechanisms of three fresh-water fishes Hughes & Shelton (1957, 1958) have substantiated the view (Woskoboinikoff & Balabai, 1936, 1937; van Dam, 1938; Henschel, 1939) that the respiratory current is maintained by the action of two pumps, one which forces water through the gills (*the buccal force pump*) and another (*the opercular suction pump*) which draws water

through this sieve. Diagrammatically such a mechanism can be represented as in Fig. 1 where it can be seen that the possible variables of such a system are at least five in number. The relative sizes of the two pumps can be varied as can the resistance of the gill sieve through which the water must pass. Furthermore, the size and nature of the mouth and the opercular opening and of their valves are also obvious variables. The classification of Baglioni was largely made before such a double pumping mechanism was envisaged and Willem (1940) has categorically denied the importance of the suction pump as described by Woskoboinikoff (1932). The present study was therefore undertaken to see whether all teleost fishes would fit into Baglioni's scheme and, if so, whether a study of the pressure relations would form a quantitative basis for his classification.

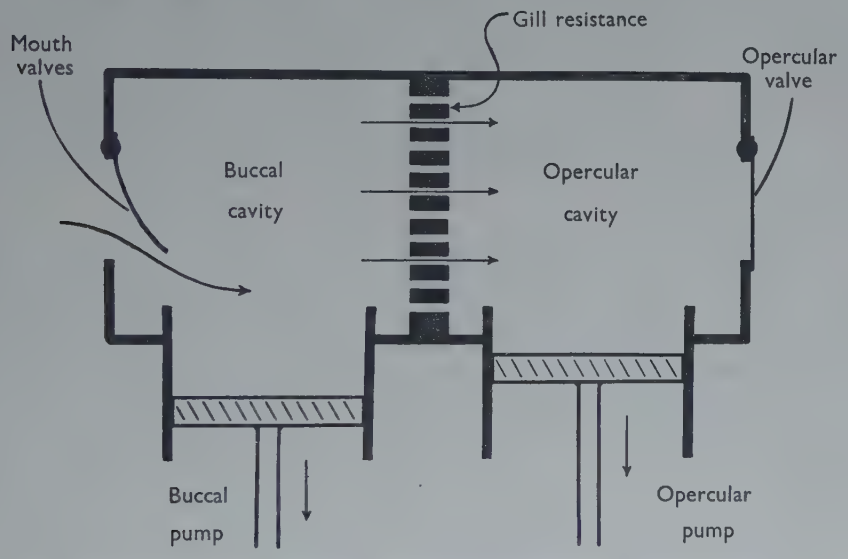


Fig. 1. Diagram to illustrate the mechanism of gill ventilation by the action of two pumps which function on either side of the gill resistance. In practice there are two opercular pumps and a single buccal pump. The openings to the buccal and opercular cavities are guarded by passive valves; variations in their size resulting from movements of the jaws and operculum have not been included. The position of the pumps is shown shortly after the commencement of inspiration.

## MATERIALS AND METHODS

The choice of fish used was largely determined by their availability in good condition for experimentation. In this respect shore-living and bottom-living species are best, as complications due to swim-bladder expansion following capture are absent or very small, but some specimens of pelagic species were also obtained. The species used were: horse mackerel (*Trachurus trachurus* (L.)), herring (*Clupea harengus* L.), whiting (*Gadus merlangus* L.), five-bearded rockling (*Onos mustela* (L.)), wrasse (*Crenilabrus melops* (L.)), bullhead (*Cottus bubalis* Euphrasén), butterfly blenny (*Blennius ocellaris* L.), grey gurnard (*Trigla gurnardus* L.), dragonet (*Callionymus lyra* L.), plaice (*Pleuronectes platessa* L.), merry sole (*Microstomus kitt* (Walbaum)),

conger eel (*Conger conger* (L.)), great pipefish (*Syngnathus acus* L.). I am indebted to the staff of the Marine Biological Laboratory, Plymouth, for the trouble they took in collecting this material.

The fish usually weighed 40–100 g. and were kept in the sea-water circulation of the laboratory. The methods used were essentially similar to those described by Hughes & Shelton (1958) but were improved in a few details. The anaesthetic M.S. 222 (Sandoz) was used as an alternative to urethane and for some species was preferred, although no significant difference could be found between recordings obtained from animals under the different anaesthetics. Most fish were held in modified versions of the clamp used previously. The fish was first anaesthetized in a more concentrated solution of the anaesthetic and after it had been fixed in the clamp it was placed in a large tank containing 25 l. of constantly aerated sea water having a much lower concentration (one-tenth) of the anaesthetic. In the case of urethane this was about 0·1 % and for M.S. 222 it was usually 1 in 50,000 or 1 in 75,000. Under these conditions the specimens continued breathing regularly while at rest and were left for about $\frac{1}{2}$ hr. before records were taken of pressure and movement. In some cases they remained in this condition for more than 3 hr. without any apparent distress.

Two Hansen condenser manometers, one utilizing a modified high-frequency circuit (Machin, 1958), were used to record the pressure in the buccal and opercular cavities simultaneously, and long levers attached to RCA 5734 mechano-transducer valves were placed lightly against lower jaw and operculum to record their movements. These four channels were fed into an Ediswan pen recorder and any two of them could be recorded simultaneously on a Cossor double-beam oscilloscope. Photographic records from the latter (Fig. 5) were used for more detailed analysis of the wave forms and also served to check any distortions due to the curvature of the pen records. The frequency response of the pen recorder (flat from 0 to 90 cyc./sec.) was conveniently the same as that of the electromanometers. In several instances ciné films were taken of the unanaesthetized animal and the respiratory current was observed by means of dyes such as methylene blue.

## RESULTS

Rather than give a detailed description of the respiration of each species studied the author has preferred to divide them into four main groups according to Baglioni's classification; the observations made on members of each group are considered together.

*Group I.* The fishes in this group were the most difficult to study mainly because for them the conditions of the experiments are inevitably abnormal, the animal being stationary relative to the water, which is very rarely the case in life. Furthermore, of all fishes pelagic species are the most difficult to obtain in good condition for experimental purposes. The results described below, however, are based upon specimens most of which were in excellent condition.

Four-channel recordings from herring and whiting (Fig. 2), and from horse mackerel, wrasse and rockling (Fig. 3) are reproduced. The frequency of the

movements ranges from 30/min. in the whiting to between 60 and 120/min. for the other four species. In the whiting the movements of the mouth precede those of the operculum by about one-tenth of a cycle. The form and frequency of the movement records are very similar to those recorded by McKendrick (1879) using unanaesthetized animals. The pressure curves (Fig. 5) show that both

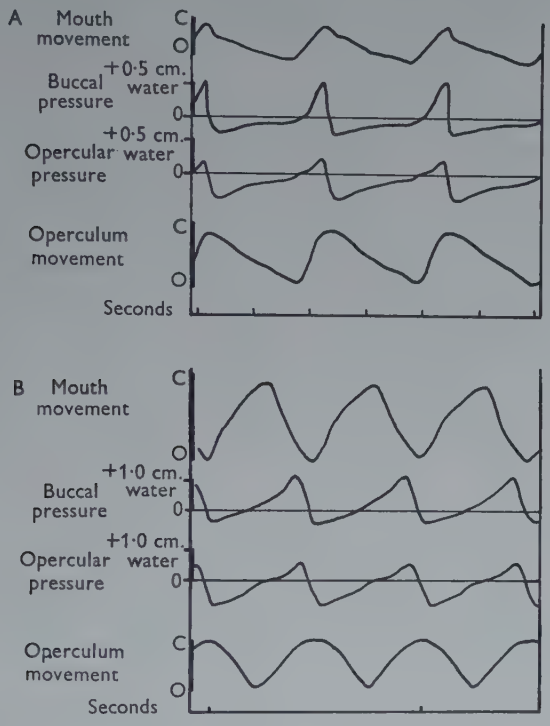


Fig. 2. Pressure changes in the buccal and opercular cavities and simultaneous mechanotransducer recordings of the lower jaw and opercular movements in: A, *Gadus merlangus* and B, *Clupea harengus*. In this and subsequent pressure recordings calibrations are shown as positive values with respect to the zero line which is the pressure in the surrounding water. Movements involved in the decrease in volume of a cavity are upwards and indicated by a 'C', whereas expansion is shown by an 'O'.

the buccal and opercular pumps are present and perhaps the latter is slightly more active than the buccal pump in producing the flow of water across the gills. This is in agreement with the fact that the phase of expansion of the two cavities is about three times longer than the closing phase. In the case of the herring these two phases are more or less equal and similarly the excess positive buccal pressure is more or less equal to the excess negative opercular pressure during the opercular expansion, suggesting that both pumps are about equally developed. The particular specimen from which this record was made was in first-class condition.

In the horse mackerel, however, although many more specimens of this species

were investigated than of the others, it was less certain that the animals were in good condition. In some cases (Fig. 3 C) the peculiarity was noticed that the operculum tended to remain in the abducted condition with the opercular valve open and was only briefly adducted shortly after the mouth had closed. The frequency of these movements was also low (10–15/min.). In such cases the pressure curves showed large positive pressures in both cavities, the buccal pressure always exceeding the opercular pressure, whereas the negative pressures recorded were relatively small. At first it was thought that these were completely abnormal specimens especially as more

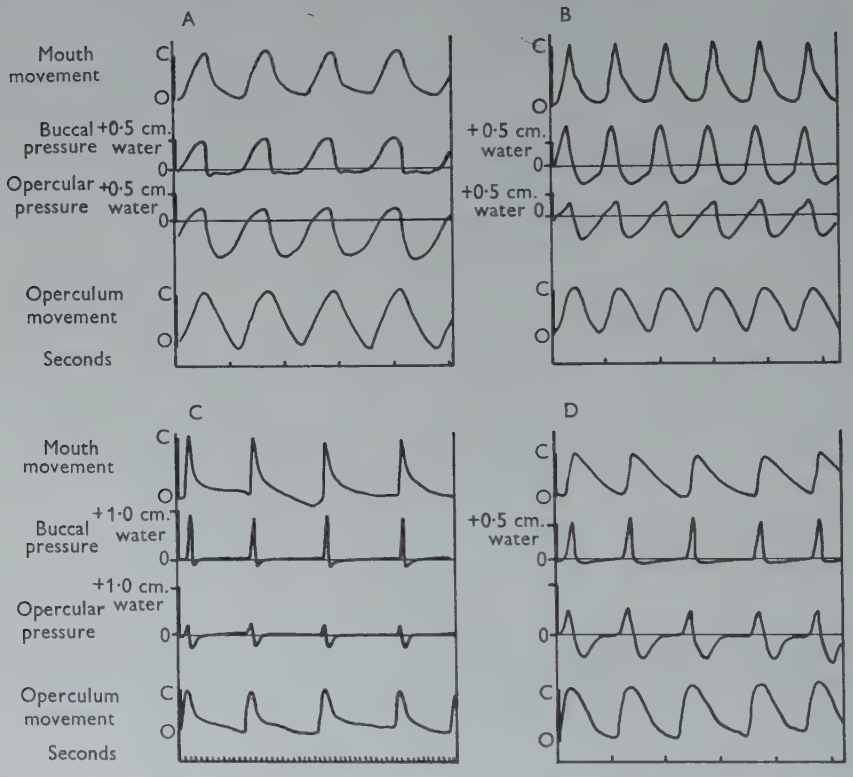


Fig. 3. Four-channel recordings of pressure changes in the buccal and opercular cavities and movements of the lower jaw and operculum: A, *Crenilabrus melops*; B, *Onos mustela*; C, *Trachurus trachurus*; D, *Trachurus trachurus*.

normal curves were later obtained—see Fig. 3 D. It can be seen in these recordings that the opercular pressure curve has a very distinct negative phase, although the buccal pressure curve still has only the sharp positive peak which occurs during the closing of the mouth. However, it is possible that the first type of record obtained is not entirely abnormal but is representative of the movements which occur during active swimming. In this case the maintained abduction of the operculum with the mouth open at the same time would ensure a continuous flow of water across the gills as a result of the animal's forward progression. Attention was drawn to this type of

ventilation by Willem (1947) in his first group which also comprised nektonic forms. He noted that each inspiration was followed by a relatively long pause during which the mouth and opercular clefts remained open. Direct observation of another carangid, the California yellowtail (*Seriola dorsalis*) at the Marineland Aquarium, Los Angeles, has confirmed this description.

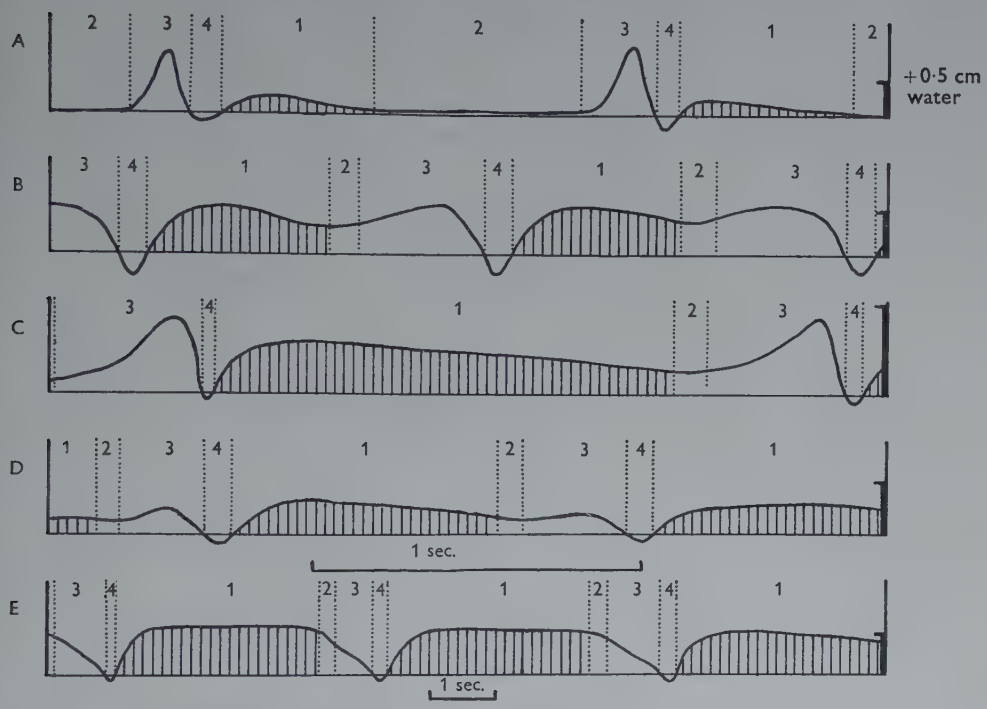


Fig. 4. Curves showing the differential pressure between the buccal and opercular cavities during the respiratory movements of: A, *Trachurus trachurus*; B, *Crenilabrus melops*; C, *Gadus merlangus*; D, *Cottus bubalis*; E, *Callionymus lyra*. A positive differential pressure indicates that the pressure in the buccal cavity is greater than that in the opercular cavities. The respiratory cycle is divided into the four phases described by Hughes & Shelton (1958). Phase 1 of the cycle when the opercular suction pump is predominant, has been shaded. Calibration pressures = +0·5 cm. water. Time scale is the same in all cases except *Callionymus*.

Another species investigated which will be included in this group is the wrasse (*Crenilabrus melops*), in which the pressure curves (Figs. 3A and 5) are very similar to those obtained from the trout (Hughes & Shelton, 1957, 1958). The frequency of movements is about 60/min., also as in the trout. The mouth begins to open about one-sixth of a cycle before the operculum abducts but closes only one-twelfth of a cycle earlier. In the pressure curves there is a well-defined transition with reversal of the differential pressure.

The differential pressure curves indicate that the two pumps are fairly well balanced in the wrasse (Fig. 4B) and herring. The buccal pump may predominate in the horse mackerel (Fig. 4A) and rockling but the opercular pump plays the more

important role in the whiting (Fig. 4C). Some differential curves also showed that there was frequently no reversal of the pressure gradient in the horse mackerel and five-bearded rockling.

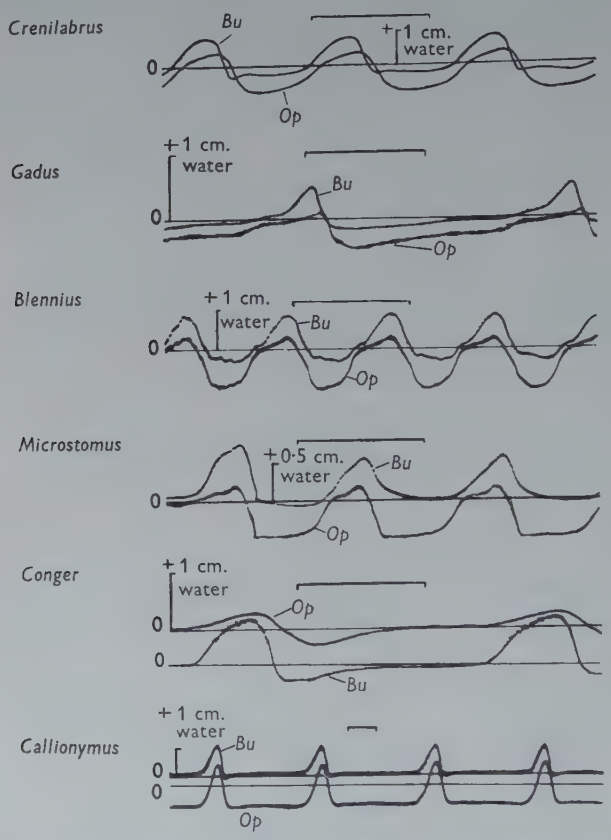


Fig. 5. Oscilloscope records of simultaneous pressure changes in the buccal (*Bu*) and opercular (*Op*) cavities of *Crenilabrus melops*, *Gadus merlangus*, *Blennius ocellaris*, *Microstomus kitt*, *Conger conger* and *Callionymus lyra*. In all except *Conger* and *Callionymus* the zero pressure is identical for both curves, but in these two cases they are shown separately. The sensitivity of both records from a single fish were equal and is shown by the calibration pressure. Time = 1 sec.

*Group II.* In the families which Baglioni included in this group the following species were studied: *Cottus bubalis*, *Blennius ocellaris*, *Trigla gurnardus*, and *Callionymus lyra*. These are largely bottom-living fishes in which the branchiostegal apparatus is very much better developed than in the preceding group of pelagic fishes. Many of the species included in this group were found to be very amenable to investigation. Details of their respiratory movements vary but a fairly definite series can be recognized which shows the increasing predominance of the opercular suction pump as the main mechanism in producing the respiratory current (Fig. 4). In some forms such as *Trigla* and *Callionymus* the exhalant current is directed dorsally from the operculum, an adaptation which will produce least disturbance of

the sand or mud of their benthic habitats. It is difficult to measure the relative volumes of the buccal and opercular cavities, but there can be no doubt that the opercular cavity is relatively much larger in most of the fish of this group. The frequency of the breathing movements is generally low, especially in forms such as *Cottus* and *Callionymus*. In the latter the frequency was 12–20/min. *Blennius ocellaris* has a more rapid rhythm of 70/min. and the positive pressure in the buccal cavity is here relatively greater than in the other species. This animal has well-marked maxillary and mandibular valves which are very effective as is shown by the marked difference in the pressure curve recorded just outside and just inside these valves (Fig. 6B). The mouth movements recorded from this species were smaller and often more complicated than in other forms, but it is possible that this is an artifact due to the position of the transducer lever. *Cottus* (Fig. 6C, D) also has maxillary and mandibular valves but here the positive pressure in the buccal cavity is accompanied by a relatively large positive pressure in the opercular cavity and hence the differential pressure during this phase of the cycle is not so marked as in *Blennius*. During the phase of opercular expansion the negative pressure in the opercular cavity is much greater than that in the buccal cavity.

In discussing the breathing of *Cottus*, Willem (1940) considered that the respiratory current was maintained solely by the action of the branchiostegal apparatus and that neither buccal nor opercular movements played an active role in the pumping mechanism. Such buccal movements as were discernible he considered to be entirely passive and due to the influence of pressure changes in the buccal cavity produced as a result of those in the opercular cavity. The present observations are not entirely in agreement with this description, but there can be no doubt that the branchiostegal apparatus plays a very important role in the production of pressure changes in the opercular cavity. In *Cottus* then, the opercular suction pump certainly plays a predominant part in gill ventilation, whereas in *Blennius* the buccal pump is still about equally important Fig. 9A. The mechanism of *Trigla* appears to be similar to that of *Blennius* in that the positive pressure in the buccal cavity exceeds that in the opercular cavity quite markedly. In *Callionymus*, however, the opercular pump is even more important than in *Cottus* and for this reason it was studied in greater detail.

Willem (1947) is the only previous author to have noted the extremely interesting mechanism found in *Callionymus* and he drew attention to the lengthening of the inspiratory phase and to its complex nature. The latter is shown in the movement records of the operculum which, as in many other cases, is rapid to begin with but continues more gradually during the phase of sustained negative pressure. He also noted the reduction in gill area found in this species which has been confirmed quantitatively (Hughes, unpublished). Some films were taken whilst specimens breathed water containing indian ink and it was found that water entered the mouth for about two-thirds of the cycle but was ejected dorsally through the restricted opening of the contracting opercular cavity during only one-fifth of a cycle. This observation fits in with recordings of the movements and pressures as shown in Fig. 7. It can be seen that there is a positive pressure in both cavities for about one-

fifth of a cycle, and that the pressure is only slightly greater in the buccal cavity than in the opercular cavity. This suggests that during this phase a complete emptying of the whole system occurs and that subsequently there is first an expansion of the

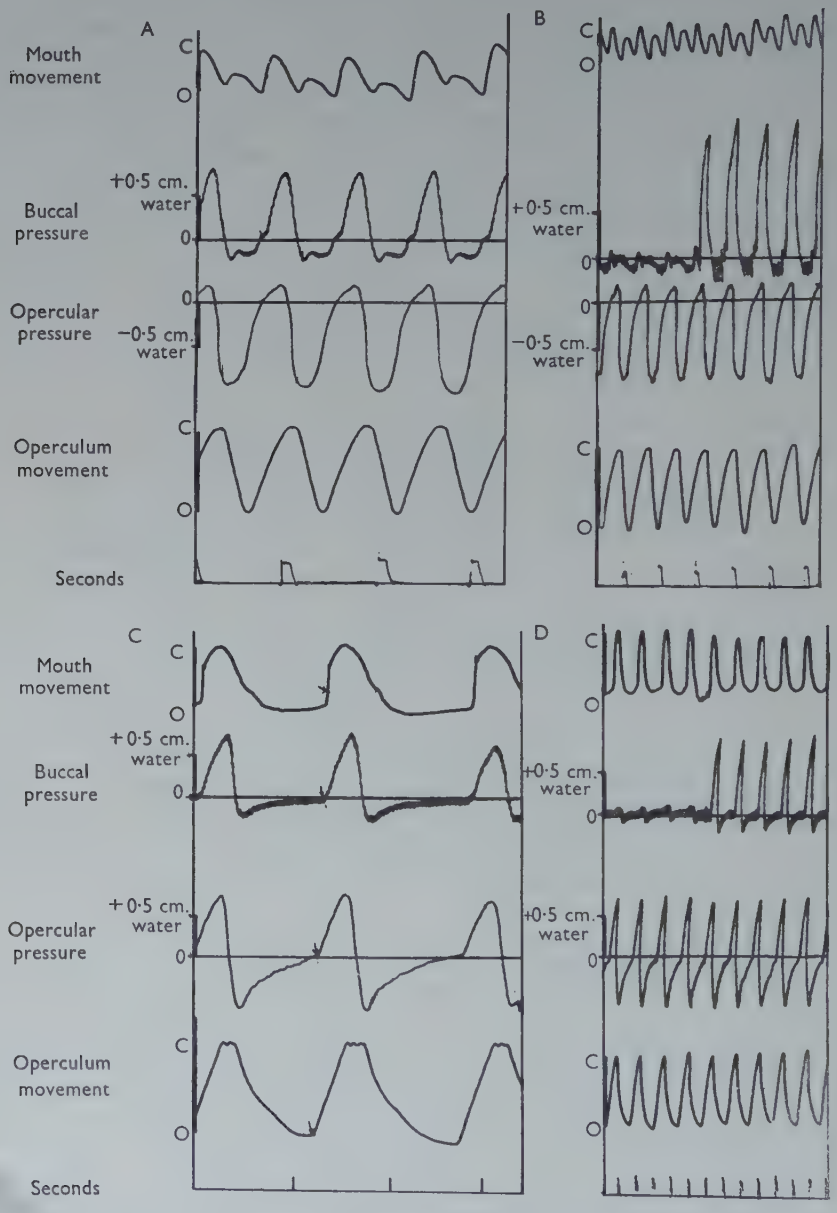


Fig. 6. *Blennius ocellaris* (A and B); *Cottus bubalis* (C and D). Four-channel recordings of movements of the lower jaw and operculum and associated pressure changes in the buccal and opercular cavities during normal respiration (A, C). In B and D one pressure-recording needle is gradually inserted through the mouth and detects the marked increase in positive pressure when it passes the maxillary and mandibular valves.

buccal cavity and then a gradual expansion of the large opercular cavities accompanied by a sustained negative pressure which draws water through the gills. The differential pressure curve (Fig. 4 E) indicates this very marked predominance of the opercular suction pump as a mechanism for drawing water through the gills. Both the buccal and opercular pumps seem to play an important role in the ejection of water from the whole system. It is possible that during the phase of ejection the gill resistance is very low so that the two cavities function almost as a single one. It was frequently found that fish of this species showed periodic departures from their normal pattern, similar to the 'coughing' found in the Cyprinidae. Thus in Fig. 7B

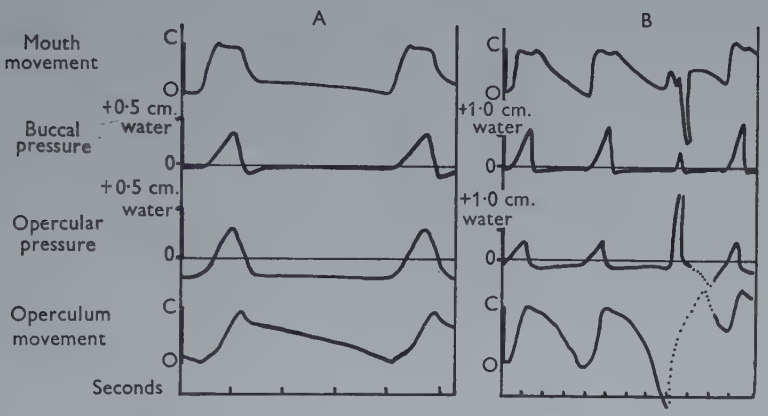


Fig. 7. *Callionymus lyra.* Respiratory movements of the lower jaw and operculum together with the associated pressure changes in the buccal and opercular cavities. In B the fish made a 'coughing' movement after two normal respiratory cycles.

it can be seen that during such departures the operculum and mouth expand more than normally after which the opercular cavity suddenly decreases in volume to produce a very large positive pressure which exceeds that in the buccal cavity, suggesting that there is a reversal in the flow through the gills during the 'cough'.

*Group III.* According to Baglioni (1907) fishes in which the branchiostegal apparatus plays the most prominent role during breathing are found in the families Scorpaenidae, Trachinidae and the order Pediculati (Lophiiformes) .These are true benthic forms as also is the family Pleuronectidae. As the latter is the only one of these four families which it has so far been possible to investigate, it will be discussed here; Baglioni classified the Pleuronectidae in Group II on the basis of their type of breathing but in Group III on the basis of their habitat.

These flatfish were not easy animals to study because of the difficulties of restraining them. The most effective method found was to fasten the fish to a brick with a light bandage so that only the head projected; by placing the fish in a vertical position it was possible to record from both opercula simultaneously. Alternatively, the fish can be placed in its normal orientation, but then only the buccal and dorsal opercular pressures can be recorded. There are wide discrepancies in the descriptions of the breathing of flatfish under normal conditions, but apart from Henschel's

(1941) work few studies have been made specifically of their respiratory adaptations. Some observers maintain that water only leaves from the lower operculum and they point out the adaptive significance of this arrangement because if it left from the upper operculum disturbances of the sand would destroy the camouflage so characteristic of many species. Other workers assert the opposite. Schmidt (1915), for instance, pointed out that normal respiration would be a difficult matter for a fish lying on its side on the bottom. Schmidt's paper is in Russian and Norman (1934) gives the following quotation: 'Not only would considerable force be required to raise the operculum of the blind side, but the action of the exhalant current of water would tend to lift the body of the fish from the bottom; further, the danger of clogging the delicate gill-lamellae with particles of sand or mud which might enter the lower branchial chamber would be a very real one.' Schmidt therefore considered that only the upper operculum is functional when the animal is at rest. His work was based entirely on preserved specimens and provides a valuable comparative account of the different types of opercular valves found in flat fish, although some of his conclusions regarding their function have been disputed by Henschel (1941).

Orcutt (1950) has described how when at rest a flounder is supported by the arching of its dorsal and ventral fins in such a way that it is held slightly away from the substratum. Plaice also do this and have often been observed using both opercula for breathing when at rest in the Plymouth aquarium. When observed in a glass-bottomed tank, dye drawn in at the mouth can readily be seen to be pumped out from both opercular cavities. As far as can be judged by this method, these observations suggest that although a greater volume emerges from the upper opercular cavity the difference is not substantial. The gill area is identical on the two sides (Hughes, unpublished) and the opercula are almost completely symmetrical, but Schmidt noticed that there are differences in the detailed structure of the valves on the two sides.

The animals certainly used both opercula under the conditions of the experiments discussed here. When movement and pressure were recorded from both opercula almost identical records were obtained from the two sides (Fig. 8B). If anything the pressures were a little less on the blind side. The rhythm is about 30/min. (*Pleuronectes*) or 60/min. (*Microstomus*). Adduction occupies one-quarter to one-third of a cycle when the pressure is positive with respect to the outside. During the longer abduction movements the pressure remains negative while water is drawn through the gills. This negative phase is particularly well developed in *Microstomus* and in contrast the positive phase predominates in the buccal cavity and occurs when the mouth is closed. Closing of the mouth precedes adduction of the opercula by about one-tenth of a cycle and the buccal pressure becomes positive before the opercular pressure. It is significant, however, that the opercular pressure begins to fall before the buccal pressure (Fig. 5). In a typical teleost such as the trout the opposite is true and the buccal pressure also becomes negative before the opercular pressure. This is the transitional phase (4) when the differential pressure shows a brief reversal in most fishes, but in both the species of flatfish investigated no such reversal has been found (Fig. 9B, C). Clearly, the opercular suction pump

predominates although the buccal pressure pump plays quite an important role especially in *Microstomus*.

The apparent absence of a reversal in the pressure gradient may well be associated with the existence of an active mechanism for closing the opercular cavity. Such a system would allow an earlier fall in pressure than is possible in one requiring the development of a negative pressure *before* the valve can close. The detailed working of this mechanism is beyond the scope of the present work and needs more detailed investigation especially in view of Schmidt's (1915) comparative account of differences in the valve structure among flat fish. The adaptive value of such a mechanism is obvious, however, for as in the skate (Hughes, 1960) it will restrict the entry of particles into the opercular cavity even if the fish is completely buried.

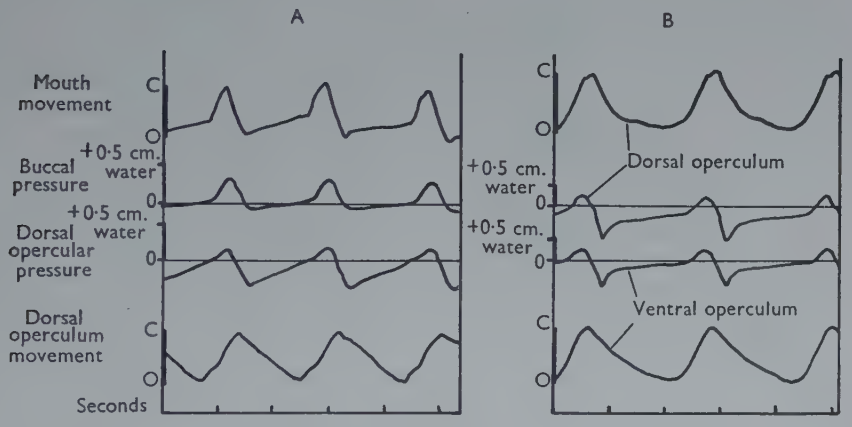


Fig. 8. *Pleuronectes platessa*. Four-channel recordings of movement and pressure recordings during normal respiration. A, Movements of lower jaw and dorsal operculum and associated pressure changes in buccal and dorsal opercular cavities. B, Movement and pressure recordings from the dorsal opercular cavity and the ventral opercular cavity.

*Group IV*. Included here are a variety of families which have lost a true branchiostegal apparatus. Baglioni (1907) recognized this as a heterogeneous group in which there is no common breathing mechanism. This is well illustrated by the two specimens investigated in the present work; in one, *Conger conger*, the buccal force pump is the more important part of the mechanism whereas in the other, *Syngnathus acus*, ventilation of the gills is largely achieved by the action of the opercular suction pump.

The recordings from the conger eel clearly show much larger pressure changes in the buccal cavity than in the opercular cavity (Figs. 5, 10B). This was unexpected at first as the opercular cavity is greatly extended posteriorly and appears to play quite an active part during breathing, peristaltic waves being seen to pass along it. However, perhaps it is better to look upon this extension as a greatly enlarged opercular valve and not as a part of the active pumping mechanism of the operculum. Mechanical recordings (Fig. 10A) showed that closing of the mouth precedes adduction of the operculum by one-sixteenth of a cycle in a fish in which the breathing

frequency was 30/min. Adduction is not simultaneous along the whole length of the opercular cavity as both films and recordings show that a wave of contraction passes backwards. The positive pressure recorded in the buccal cavity is much larger than that in the opercular cavity during these movements. During the expansion phase, however, abduction first occurs in a region about two-thirds along the opercular cavity. This region changes shape most rapidly especially during the change-over

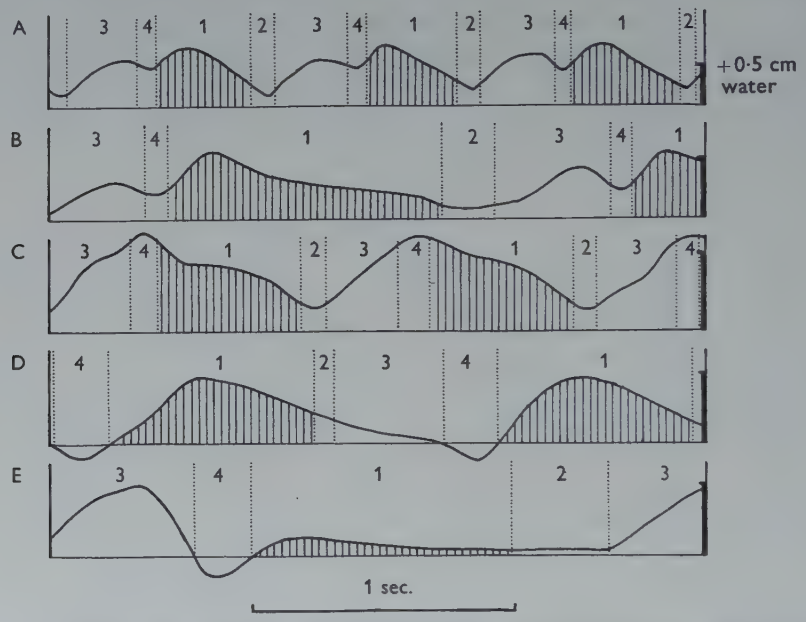


Fig. 9. Time-course of the differential pressure between the buccal and opercular cavities during the respiratory movements of A, *Blennius ocellaris*; B, *Pleuronectes platessa*; C, *Microstomus kitt*; D, *Syngnathus acus*; and E, *Conger conger*. A positive differential pressure indicates that the pressure in the buccal cavity exceeds that in the opercular cavity. Notice the absence of any phase when the pressure differential is reversed in the first three curves. The respiratory cycle is divided into four phases based on those described by Hughes & Shelton (1958). Phase 1, when the opercular suction pump predominates, has been shaded. Calibration pressures = +0·5 cm.

from adduction to abduction (Fig. 10A). Expansion of the buccal cavity probably takes place before the earliest abduction of the operculum but this is not always obvious from the movement records. The fall in pressure in the buccal cavity is quite large immediately the mouth opens, but soon the opercular cavity becomes more negative than the buccal cavity and water is drawn across the gills.

The differential pressure curve (Fig. 9E) clearly shows that the buccal force pump is the main mechanism causing water to flow over the gills and that the phase of the opercular suction pump, although longer than that of the buccal force pump, is of relatively little importance. The presence of the backward extension of the opercular cavity serves to damp out any pressure changes in it and also, as was pointed out by Willem (1947), will diminish variations in the initial speed of the respiratory current and so produce little disturbances of the medium. Such a mechanism probably is

adaptive in a form with reduced paired fins; in other fishes the paired fins are often used to back-paddle when the fish is stationary in order to counteract the slight propulsive effect of the exhalant stream from the operculum. The only occasion on which relatively large negative pressures were recorded from the opercular cavity was when the fish was firmly biting the recording needle in its mouth and hence movements of the latter were smaller than normal. It is therefore possible that the opercular suction pump becomes of greater importance during feeding than is indicated by the records of Figs. 5, 10B. Another variation was the occasional

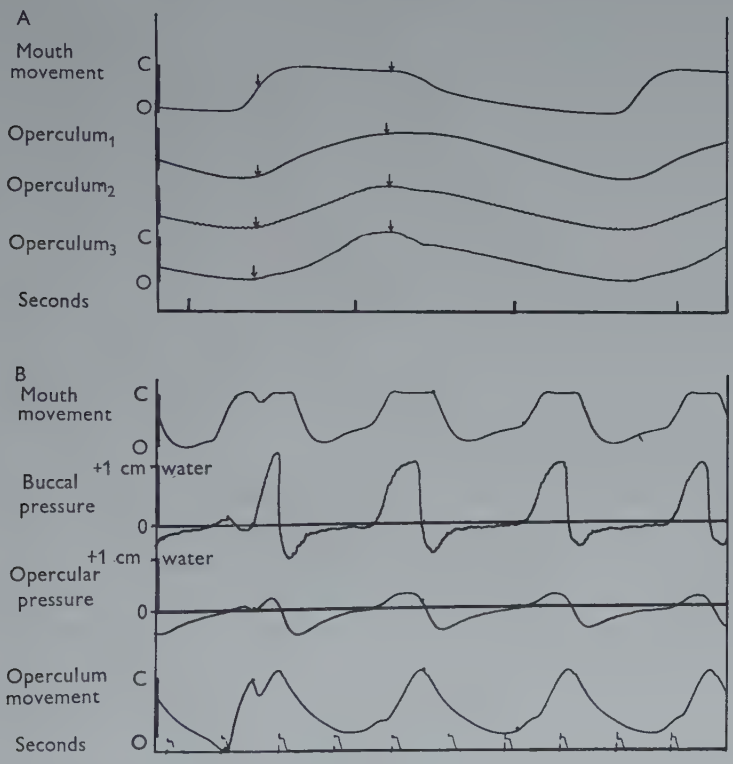


Fig. 10. *Conger conger*: A, Mechanotransducer recordings of respiratory movements of the lower jaw and three positions on the opercular cavity, of which $Operculum_1$ is the most anterior. The arrows mark identical instants on the four curves. B, Four-channel recording of pressure changes in the buccal and opercular cavities associated with movements of the lower jaw and operculum.

'coughing' behaviour which occurred about once every twenty cycles. As shown during the first cycle of Fig. 10B the operculum is held wide open while the buccal cavity decreases in volume, but it then adducts quite rapidly. During these movements there are relatively small pressure changes, but after a slight expansion the buccal cavity decreases in volume still further and its pressure rises sharply. The operculum also has an extra abduction and then it closes before expanding gradually. The negative pressure in the buccal cavity is slightly greater than normal but that in the opercular cavity remains unchanged. During the succeeding respiratory cycle there is a prolongation of the positive phase in both cavities.

In contrast to the predominance of the buccal pressure pump in the conger eel is the predominance of the opercular suction pump in the pipefish. Even with quite a large specimen it was impossible to hold the animal stationary and to record both movement and pressure without excessively interfering with normal respiration. It was not possible to record movements but the differential pressure curve derived from the pressure records show quite clearly the predominance of the opercular suction pump (Fig. 9D). This is of interest for, as mentioned above, there is no branchiostegal apparatus in this group. The opercular cavity, however, is relatively enlarged because of the well-developed operculum and it is closed except for a small dorsal aperture through which the water is ejected. Very small pressure changes were recorded from the mouth, as might be expected because of the relatively slight movements of the lower jaw. The narrow mouth together with the reduced opercular pressure produces a rapid flow into the mouth which may be used in feeding.

Leiner (1938) has pointed out the importance of the active opercular suction in the breathing and also in the feeding of the sea-horse, another member of the Syngnathidae.

## DISCUSSION

This comparative survey of gill ventilation in marine teleosts has confirmed the results obtained previously in three fresh-water species (Hughes & Shelton, 1958). The opercular and buccal pressure curves have essentially the same form in all the species so far investigated and demonstrate the general validity of the view that water passes across the gills by the action of a buccal pressure pump in front and of opercular suction pumps behind the gills. The results reported above show that differences in the relative importance of the pumps occur in various fishes although they all utilize this same basic mechanism. It has been found that the differential pressure curve gives a good indication of these differences, despite the possible influence of variations in gill resistance which may occur during a respiratory cycle (Hughes, 1960). The pressure curves show the overall effect of the pumping mechanism, however, and give little information about the relative importance of the constituent parts of the pumps. The action of the suction pump, for instance, depends upon the co-operation of movements of opercular bones and of the branchiostegal apparatus, both of which produce a reduction in the pressure recorded from the opercular cavity. Thus while in general it is found that the suction pump becomes more important in species with an increased development of the branchiostegal rays this is not necessarily the case because the opercular bones may be correspondingly reduced. Nor is the converse true, as is well illustrated by the pipefish in which the suction pump is very well developed but the branchiostegal apparatus is absent.

It is clear, however, that the degree of development of the opercular suction pump is one of the main variables between fish of different habitats. This finding correlates very well with the classifications of fish respiratory movements (Baglioni, 1907; Willem, 1947; Bertin, 1958) that have been based almost entirely on morphological features. The correlation between the habitat of the animals and the physiology of their ventilation mechanism has now been demonstrated and indicates further prob-

lems to be studied. The adaptive value of an increased development of the suction pump in benthic forms is fairly clear when it is remembered that most of the time they live in water which is almost stationary. Selection has favoured the evolution of a mechanism which is well adapted to drawing a current across the gills during a relatively long part of the respiratory cycle. Such mechanisms are admirably adapted to ensure a steady flow across the gills without creating any disturbance of the muddy or sandy bottom. On the other hand, the buccal pump operates with the opercular pump during a very brief period to produce a rapid evacuation of most of the respiratory system. Thus in this part of the cycle the de-oxygenated water is ejected at high velocity often through a narrow, dorsal and posteriorly directed opening. The adaptations of sponges to produce such a jet and so decrease fouling of their own water have been discussed by Bidder (1923) and his concept of a 'diameter of supply' might well be applied here. At the other extreme it is not surprising that fishes which lead an active life and mainly swim upstream in flowing water should not possess such adaptations.

As stated in the introduction, most previous classifications of teleost respiratory movements are fundamentally the same, but there are certain details in which they differ and some of the present work is of interest in this connexion. For instance, both Willem and Bertin classify *Callionymus* and eels in the same group, whereas we have seen that the former is an excellent example of a fish with a dominant suction pump, whereas at least in the conger eel the opposite is true. Baglioni's system seems best in this respect although it involves the creation of a heterogeneous group (IV) of species with reduced branchiostegal apparatus. Willem also creates a special group including some nektonic forms like the mackerel and some salmonids and cyprinoids which swim continuously and make few definite respiratory movements but vary the flow by changing the size of the mouth opening. This distinction is a fine one and is of the same order as that between Baglioni's Groups II and III. What is quite apparent from all attempts at classification is that there is a continuous spectrum of forms between types such as the mackerel through the trout and wrasse to the bullheads, dragonet and flatfish.

There are, however, several interesting features which the pressure curves have revealed and which previously have not been noted. On several occasions, for instance, it has been observed that the differential pressure curve does not include a transitional phase with a reversal in the gradient. This has been found several times in the plaice, merry sole and blenny, but also occasionally in the herring, rockling and horse mackerel. Its existence in the two flatfish is almost certainly correlated with an active branchiostegal valve mechanism and it is hoped that a detailed study of this will be of assistance in interpreting this phenomenon in other species. A further point of interest in the curves is provided by the variations found in the differential pressure in relation to the maximum absolute pressure change in one of the cavities. Thus during the buccal pump phase of the dragonet the maximum buccal positive pressure is as great as the maximum negative opercular pressure during the opercular pump phase. The differential pressure, however, is very much less during the former phase, because the pressure in the opercular cavity is also quite large and

the two curves are very close to one another. The converse is true in many specimens of tench (Hughes & Shelton, 1958) where the two pressure curves are almost identical during their negative phases. Such observations suggest that variations in gill resistance occur during the respiratory cycle and that their nature depends on the species of fish. It is clear from this discussion that more detailed information is required about the properties not only of this resistance but also of the other two resistances shown in Fig. 1, i.e. those of the mouth and opercular openings and their valves.

## SUMMARY

1. Movements of the lower jaw and operculum have been recorded simultaneously with the associated pressure changes in the buccal and opercular cavities during breathing of the following species: *Trachurus trachurus* (L.), *Clupea harengus* L., *Gadus merlangus* L., *Onos mustela* (L.), *Crenilabrus melops* (L.), *Cottus bubalis* Euphrasén, *Blennius ocellaris* L., *Trigla gurnardus* L., *Callionymus lyra* L., *Pleuronectes platessa* L., *Microstomus kitt* (Walbaum), *Conger conger* (L.), *Syngnathus acus* L.

2. In all species ventilation of the gills is achieved by the action of a buccal pressure pump and of opercular suction pumps.

3. The time course of the pressure changes indicates differences in the relative importance of the two pumps which are related to the habitat of the fish. The suction pump becomes of greatest importance in fishes which spend most of their lives on the sea bottom.

4. In several species the differential pressure curve does not include the brief period of reversal in pressure gradient found in most fishes so far investigated. Notable among these species are the two flatfishes investigated and in which there is some evidence for an active opercular valve.

5. In general, the results confirm the validity of Baglioni's classification of the respiratory mechanisms of teleost fishes.

I wish to thank the Director and staff of the Plymouth Laboratory for their assistance and for the facilities they provided during the course of this work.

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# NERVOUS CONTROL OF MOVEMENT IN ANNELIDS

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## INTRODUCTION

The stage has been set for the analysis of neural control of movement in nereids by the thorough description of the nervous system made by Smith (1956, 1957). Previous descriptions of nervous and muscular histology were provided by Quatrefages (1850), Retzius (1895), Hamaker (1898), Langdon (1900) and Prenant (1929). But details of the histology of individual fibres are lacking.

The involvement of giant axons in muscular activity of annelids is a special case which has received some attention (Nicol, 1951; Horridge, 1959). The giant fibres are either internuncials as in nereids and earthworms or final motor neurons as in some sabellids. They cause synchronous contraction of large areas of musculature with muscle action potentials which are already maximal for the first impulse. Smaller contractions than those caused by the giant fibres occur, probably in the same muscles, but these have not been correlated with the activity of single nerve cells.

Only a limited body of knowledge exists on the neuromuscular relations in non-giant systems. Buddington (1902) found two types of contraction when stimulating body-wall strips of earthworms, a fast spike-like contraction and a later slower wave, both showing summation with repetition. Botsford (1941) found facilitation in such strips. The presence of a functional nerve net affecting the muscles of earthworms was argued by Prosser (1935, 1946), and spread of excitation in leech body wall was recorded by Schwab (1949). Some information about the role of the individual segmental nerves in movement has been gained by Gaskell in leeches (1914), by Horridge in polychaetes (1959), and by Horridge & Roberts in earthworms (1959). Maxwell (1897) suggested a reflex function for the parapodial ganglion of polychaetes.

The present study extends our knowledge by demonstrating slow and fast nerve-muscle systems and something of their distribution. It argues against a nerve net and for a reflex function of parapodial ganglia.

## EXPERIMENTS ON POLYCHAETES

Results on polychaetes were obtained during the summer of 1958 at the Friday Harbor Marine Laboratory of the University of Washington. Clamworms, *Neanthes brandti* (Malmgren), were collected in the pebbly mud of False Bay, San Juan Island. These were kept for not over 1 week in the open sea-water system; if kept longer, they lost vitality. Water temperature was about 14° C.

*Neanthes virens* Sars, sent from the Marine Biological Laboratory, Woods Hole, Mass., were studied in Los Angeles.

### *Motor functions of the segmental nerves*

*Preparation.* The most useful dissection consisted of the following. Specimens of 50 cm. or greater length were used. A 4 or 5 cm. piece from the wider anterior end of the worm was selected and pinned ventral side up in a wax dish. A median ventral incision was made through the cuticle which was then pulled laterally to expose the ventral musculature and segmental nerves. The segmental nerve selected was lifted on paired fine steel electrodes until an insulating air space formed under it. The region was then covered with mineral oil in order to prevent desiccation and maintain insulation. A few test pulses were delivered to determine whether the preparation was responsive. If a muscular response occurred, the proximal end of the nerve was cut in order to eliminate reflex activity.

Only a small percentage of the preparations made in this way showed activity. It was usually necessary to pick up several segmental nerves in succession in order to obtain a response in the segment to which the nerve belonged. More often stimulation of a segmental nerve still connected centrally resulted in movement in other segments. It is presumed that stretching during the removal of the cuticle and lifting of the nerve resulted in damage to many fibres. But if it was damage which caused the low probability of a responding preparation, it may also have been responsible for the ease with which all-or-none preparations were obtained. Since there are only a few motor fibres in each nerve (Smith, 1957) (Fig. 1) the apparent destruction of some of these left nerves with only one or two modes of action. Those preparations which did show activity continued to do so for a good part of an hour.

Muscle action potential recordings were made through a tapered tungsten probe insulated to the tip and placed on the muscles, therefore recording extracellularly from many units in a small area. The worm was grounded at a remote, inactive region. Temperature of the sea-water bath was from 14° to 22° C. Qualitative changes in response did not occur in this range. Entire worms showed normal locomotory activity at room temperature.

#### *Effects of stimulation*

*Nerve I.* Stimulation resulted in abduction of the parapod from its resting position against the body wall. The response occurred above a single threshold. Repetitive stimulation caused a larger movement. No electrical recordings were made.

*Nerve II.* The largest segmental nerve, the parapodial, was the easiest to prepare. Using single stimulating pulses, an all-or-none movement was produced in the innervated parapodium above a threshold intensity. Interference by the recording electrode usually prevented observation of the character of the movement. The only clear cases were adduction, but it may be expected that other components or alternatives in different cases occurred as well. Increase in stimulus intensity above this threshold usually caused no change in the response, even though several motor fibres are probably present. The single mode of action indicates that all but one motor fibres were inactive.

Repeated stimuli augmented the mechanical response. A single stimulus produced a small electrical wave of 12–15 msec. duration in the parapodium. The latency from stimulation time was about 6 msec. for a conduction distance less than 0·5 cm. A second stimulus 0·1 sec. later resulted in a somewhat larger response. At frequencies above 10/sec. the response is augmented on successive stimuli, reaching a maximum after three or four shocks. The response to the second stimulus was about 2–3 times greater than the first and that to the third was twice as large as the second. At frequencies greater than 60–75/sec., summation of the individual

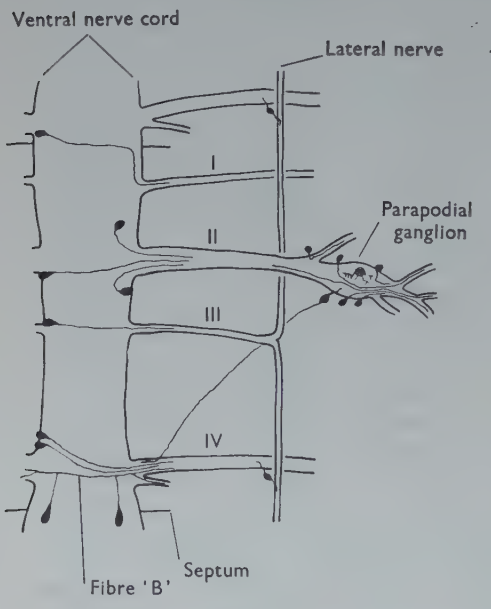


Fig. 1

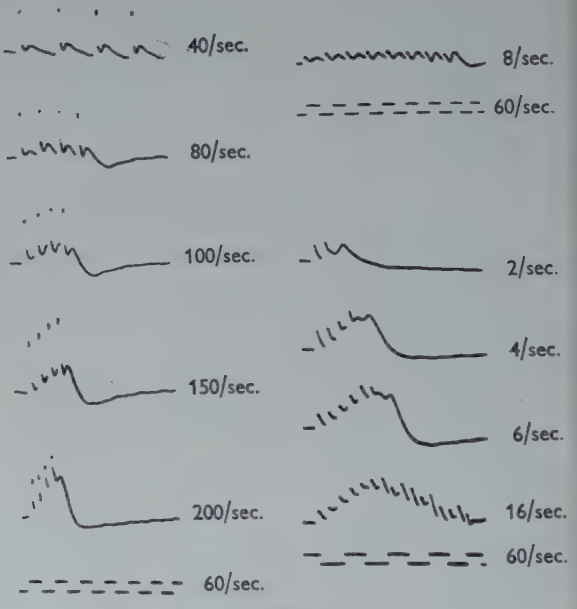


Fig. 2

Fig. 1. Nerves and ganglia of a segment of a nereid polychaete. The motor fibres and some peripheral internuncial fibres are shown. Modified from Smith (1957).

Fig. 2. Electrical response of the intrinsic parapodial musculature to stimulation of an all-or-none unit in nerve II of *Neanthes*. Left. Effect of four shocks at frequencies of 40, 80, 100, 150, 200/sec. The small muscle action potentials are preceded by large stimulus artifacts. Some facilitation is seen. Summation begins below 100/sec. At 200/sec. the fourth response is smaller than the third. Right. Top: effect of 8 shocks at 75/sec. Shows facilitation only. Bottom: effect of 2, 4, 6, 16 shocks at 125/sec. Summation is followed by early fatigue. Time markers, 60/sec.

responses occurred. Fig. 2 shows the degree of facilitation and summation to a series of four shocks delivered at different frequencies. Each record is taken during a series of bursts of four impulses repeated at 1 sec. intervals. In this way irregular changes associated with very low frequency stimulation and slow movement artifacts were avoided. Fig. 2 shows also the effect of increasing numbers of stimuli at one frequency, 125/sec. The maximum response is reached after four stimuli. A plateau level is maintained for two or three stimuli, after which the response wanes. At a lower frequency where no summation occurs, 75/sec. in this case, this waning is not noticeable with up to eight repetitions (Fig. 2).

*Nerve III.* No motor responses were found to result from stimulation of nerve III.

*Nerve IV.* Stimulation of nerve IV resulted in action potentials in the dorsal longitudinal musculature. The connexions to the ventral musculature were presumably destroyed during the dissection when lifting the nerve away from the body. Two kinds of response were elicited by stimulation of nerve IV. Only in a few cases were both found in the same preparation. In these cases the two had different threshold values and different latencies.

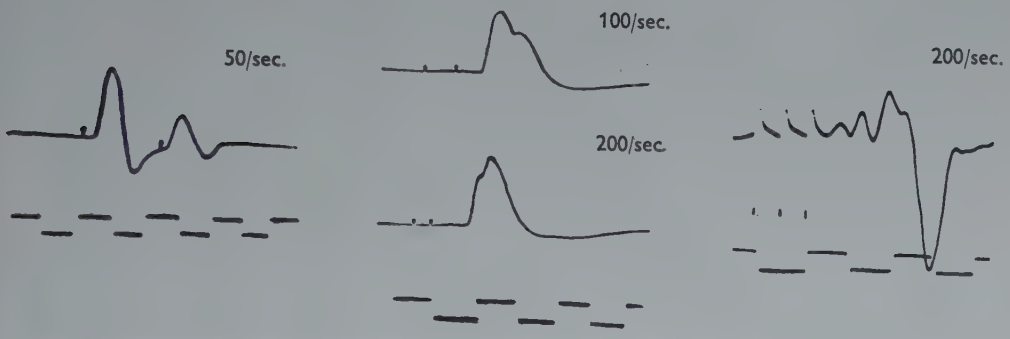


Fig. 3. Electrical responses of the dorsal longitudinal musculature to stimulation of all-or-none units in nerve IV of *Neanthes*, showing fast diminishing, rather than facilitating type of response. Left and middle: Pairs of shocks at 50, 100 and 200/sec. Right: Three shocks to a slow fibre at 200/sec. producing three facilitating responses and a fourth, large anomalous deflexion. Time markers, 60/sec.

The latency from stimulus to the *first response* is about 8 msec. for a conduction distance of under 1 cm. This is about four times as great as the latency between giant fibre spikes in the cord and muscle action potentials in the ventral musculature as recorded by superficial electrodes near the cord. The first response differs from that found in nerve II stimulation in its larger size and lack of facilitation. Except at excessively high frequency each shock is followed by a large muscle action potential of about 12 msec. duration. Each successive response even at very low frequencies (1/sec.) is smaller than the preceding one. The magnitude falls to about one half for the second shock at 1/sec. and then declines more slowly to a fairly steady low level. At frequencies above 100/sec. the individual responses fuse, but the second is still smaller than the first (a downhill *treppe*) until at above 200/sec. the second peak is higher although the increment it adds is very small (Fig. 3). It is questionable whether this uphill *treppe* could be obtained in a perfectly fresh preparation.

The *second response* in the dorsal longitudinal musculature occurs after a latency of 12–18 msec. (in different preparations). It is smaller than the fast response and shows the same summing and facilitating characteristics as the parapodial excitation. At 1/sec. this response has a constant amplitude and a duration of about 6–10 msec. In one preparation a peculiar response was noted at 200/sec. A maximum was reached on the second or third shock after which, without a fourth stimulus, another

wave of electrical activity occurred having a magnitude about 5 times larger than the preceding one and a reversed polarity (Fig. 3). This sequence was repeated about 10 times in this preparation. Without measurements of trans-membrane potential such a phenomenon is difficult to interpret, especially the reversal of polarity.

It seems justified to name the two kinds of response to nerve IV stimulation after the fast and slow responses in arthropods, the former being the diminishing, the latter the facilitating ones.

Attempts were made to record nerve action potentials from the segmental nerves of *Neanthes*. Some spikes were seen in centrally connected nerves, but these were small and not individually identifiable.

A few observations were made on other polychaetes. Giant axon potentials and associated muscle action potentials were recorded in four sabellid genera, *Eudistylia*, *Schizobranchia*, *Pseudopotamilla* and *Myxicola*. In all of these, rapidly decreasing spikes were found similar to the fast responses in *Neanthes*. The time between nerve and muscle spikes in *Myxicola* was about 3 msec. This supposedly includes no central synaptic delay, but only conduction and neuromuscular transmission time. In *Neanthes* the delay was no larger, but since the muscle potential drops out suddenly from its low-level fatigued value, while giant axon potentials are still present, an intermediate synapse is indicated (see Horridge, 1959). Apparently, latency from giant axon spike to muscle action potential will not give good information about the number of junctions between them.

The effects of stimulating other than giant fibres were not observed in these sabellids. However, slower contractions can be seen in them, especially in the cases of peristaltic waves in the body wall and slow shortening of the whole animal.

Slow contractions of the longitudinal muscle were observed to result from electrical stimulation of the cord and segmental nerves of *Aphrodite*. Tension developed very slowly with long trains of stimuli.

### *Function of the parapodial ganglion*

#### *Effects of electrical stimulation in the parapodium*

In an attempt to evaluate the role of the parapodial ganglion in either centrally controlled movements or in peripheral activity, electrical stimulation within the parapodium was effected by means of a pair of fine steel electrodes. Movements were observed visually with the aid of a microscope. Both in *Neanthes* and *Glycera* stimulation within the parapodium of intact specimens resulted in contractions both in that parapodium and elsewhere. The movements were not regularly reproducible and continued for seconds after the stimulation in many cases. They appeared not to involve simple reflexes, but either complicated patterns of reflexes or general activation. Stimulation in the parapodia of body-wall strips or worms from which a small ventral strip including the cord was removed resulted in movements in that parapodium and adjacent body-wall muscle only. Each shock produced a twitch, and repetitive stimulation produced a larger contraction. No signs of after-discharge were seen in these cases of stimulation of centrally disconnected parapodia.

This kind of stimulation must be expected in different cases to excite either motor or sensory fibres or both. The results resembled the effects of stimulation of motor fibres in nerve II in their one-to-one relationship to the stimulus. This experiment gave no evidence that the parapodial ganglia have interconnexions between segments other than through the ventral cord. The same results were obtained in experiments on *Glycera*.

*Effect of calcium-free medium on parapodial ganglion*

A strip of cuticle was dissected free from underlying tissue on the ventral side of *Neanthes* so as to carry with it the parapodial ganglion and nerve II. This was held by pins as far as possible from muscular tissue and in a position such that fluid would not flow from the ganglion toward the muscle. A small drop of isotonic sodium citrate was placed around the ganglion and reactions of the parapodium observed. In eight of twenty-four trials movements of the parapodium occurred after a few seconds. The reaction could not be obtained twice on the same preparation even after washing with sea water. It is probable that this dissection was very damaging and the low percentage of positive results was not unexpected. The movements which occurred were clonic or tetanic and clearly reflected multiple discharge in the nervous system.

*Reflexes in the isolated parapodium*

The experiment of Maxwell (1897) was repeated with some modification. Entire single parapodia, including most of nerve II, were isolated and pinned to a wax dish. These showed spontaneity for a few minutes only. Chemical stimulation with weak acetic acid placed on the cuticle so that none reached exposed muscle tissue elicited strong and prolonged contractions. Tactile stimulation, either brief taps or brushing contact with a small needle, produced local contractions in various parts of the parapodium often not near the site of stimulation. Definite patterns were observable, stimulation at a certain point always giving the same response. Several kinds of response were observed. Extension of the setae was slow and long lasting. Flexions and retractions of other parapodial structures were relatively fast and not maintained but characterized by rapid relaxation. Some of the longer lasting reactions were in the nature of a low-frequency clonus lasting around 1 sec., the individual twitches barely differentiated and full relaxation following rapidly after the last twitch. The frequency of discharge appeared to be of the order of 10/sec. Such repetitive reactions sometimes followed the briefest possible manually produced stimuli. This kind of contraction appeared identical in time-course to that which is produced by electrical stimulation of nerve II. It differed in the very small amount of muscle tissue involved compared to that excited by a single stimulus to a unit in the second nerve. None of the reflex activity found here persisted in parapodial isolates in which the parapodial ganglion was not included.

The above results suggest strongly that there are *motor neurons in the parapodial ganglion* which are involved in reflexes confined to that ganglion. Their role in these reflexes is probably not a simple relay one, but an integrative one. The responses to

brief stimuli suggest that after-discharge in the ganglion rather than prolonged activity of the sensory structures is responsible for the tetanic nature of some of the contractions. It is unlikely that all of the reflex activities of isolated parapodia could be produced by ephaptic transmission at the cut nerve end in view of the regularity and predictability of the responses. Some patterns of organization are apparent from specimen to specimen. Very local reactions may involve axonal reflexes or direct sensory-motor connexions, but these doubtless do not explain the more distant reactions which require the presence of the branches and ganglion of nerve II.

## EXPERIMENTS ON LEECHES

Medicinal leeches said to be obtained from Spain were purchased through a Los Angeles drug store. These were of the genus *Hirudo*, probably *H. medicinalis* L.

### *Motor function of segmental nerves*

Muscle action potentials in the body wall were recorded during stimulation of the peripheral stumps of segmental nerves. Stimulating pulses were routed through the forceps which held the nerve up in a mineral oil bath. The recording was through a fine insulated needle. Single pulses of sufficient amplitude produced slight visible responses. Repetition at least 3 times in 0·1 sec. was necessary to produce contractions similar to those of normal, active worms. Tetanus:twitch ratio was many hundreds to one. The electrical response to single pulses was graded with intensity without clear steps, indicating numerous motor axons in each nerve. For repetitive bursts some facilitation occurred at frequencies greater than 10–15/sec. The maximal facilitation for a second shock resulted in a muscle action potential about twice as large as that resulting from the first. Facilitation occurred for the first two or three shocks only. The individual muscle action potentials had a duration of about 25 msec. and fusion began at a stimulation rate of about 40/sec. A steep *treppe* occurred at higher frequencies. With long bursts of stimuli a high plateau was maintained for a considerable fraction of a second with slow fatigue thereafter. The muscle action potentials resulting from segmental nerve stimulation in the leech were generally similar to those of the slow system of polychaetes.

### *Peripheral spread of excitation*

The visible response of the muscle to stimulation of a segmental nerve was checked for spread of excitation. Segments adjacent to the one stimulated were separated from the central nervous system by section of the peripheral nerve roots. The response to bursts of stimuli had the appearance of spreading, with greater area responding to higher frequency or longer duration of burst. It is admitted that this spread could have been illusory. The maximum area which could be excited to contract was no greater than that measured by eight annuli. There were five annuli per segmental ganglion in the body region used.

Isolated strips of body wall containing no ventral nerve cord were stimulated with a pair of electrodes about 1 mm. apart and the resultant contractions observed with

the aid of a dissecting microscope. The area of contraction increased with increase in either frequency or duration of burst. At 20–50/sec. maximum spread was obtained within 1 sec. With the electrodes at one end of the strip the area excited never covered more than 8 annuli. The edge of the contracting field was sharply defined; a resting annulus was clearly distinguishable from an adjacent active one. The extent of spread was independent of voltage up to more than ten times threshold.

*The spread of excitation found does not require a nerve net* for explanation and such a net is contra-indicated by the sharply limited nature of the zones over which spread takes place. The spread probably takes place only among muscle fibres under the influence of the same central motor neuron, or along the muscle fibres themselves (Schwab, 1949). The appearance of a response in a region only after many stimuli may indicate that few motor fibres innervate both that region and the stimulated region and repetition is necessary to make the response visible, or that conduction along individual muscle fibres is decremental.

## DISCUSSION

### *Correlations with anatomy*

Physiological properties have been identified for several of the peripheral motor elements of the nereid nervous system described by Smith (1957). He finds only one motor axon in nerve I and this axon innervates the oblique muscles of the parapodium. An all-or-none motor response of the parapodium is associated with stimulation of nerve I.

Smith lists three axons in nerve II presumably innervating only the intrinsic muscles of the parapodium. Movements and muscle action potentials in the parapodium follow stimulation of nerve II. Separate responses which could be correlated with the three axons were not found. The possibility that these axons do not directly innervate the muscles, but relay in the parapodial ganglion, was pointed out by Smith. Since the responses in the parapodium follow stimulation in a one-to-one manner the proposed relays would have to be of a non-integrating type except for the possibility of spread to increased number of units. No evidence for spread with repetition was found. Some axons pass directly through the ganglion and these could account for the one-to-one responses.

The cells of the parapodial ganglion must be involved in the reflexes found by Maxwell (1897) and Horridge (1959). If two-way conduction is presumed in the axons which pass through the ganglion then these cannot be involved in very local activity, since they must have large fields of innervation, and we may conclude that only the motor cells of the parapodial ganglion are involved in local reflexes. Most likely candidates for this role are the unipolar 'interneurons' recognized by Smith which send processes into the branches of nerve II (Fig. 1). The long reactions which sometimes follow brief stimuli suggest that some integrative functions occur in the ganglion. A part of these may tentatively be assigned to the multipolar interneurons of the ganglion. The peripheral motor neurons (Fig. 1) could be used

in the case of centrally controlled movements as well. If so, then there would either have to be two sets of them or each would receive two kinds of input, one from central neurons and one from local sensory or internuncial neurons. Overlap of functions is necessary at some point in this system if the same muscles are to be used in both centrally and peripherally controlled movements. Since peripheral motor relays do not seem to be present in other muscle fields of this animal (Horridge, 1959) the most reasonable hypothesis is that the parapodial ganglion functions only in a local reflex system which is not under central influence and that the same muscles receive separate innervation from the nerve cord. Alternatively, it is possible that the overlap begins at the ganglion with an avalanching relay from nerve II motor fibres.

Smith finds one, or perhaps two, motor axons in nerve III. No motor responses were found associated with this nerve, but it is the smallest nerve and few preparations were made from which results could have been expected.

Nerve IV contains three motor axons. The largest, Hamaker's 'fibre B', has been shown by Horridge to receive input from the lateral giant fibres and to innervate the longitudinal muscles. In *Harmothoë*, according to Horridge, this fibre is unique in supplying directly both the dorsal and ventral longitudinal muscles. He found that a second axon also innervated the ventral muscles. In the present study two kinds of responses were identified in the dorsal longitudinal musculature. If *Neanthes* is homologous to *Harmothoë* in respect of innervation of the longitudinal muscles, that is, if only one fibre is common to both dorsal and ventral strips, then all three of Smith's nerve IV motor axons are accounted for (Fig. 1). The large pair is connected so that synchronous contractions of dorsum and ventrum on both sides are produced. The smaller fibres must be so arranged as to allow the alternate activity of the two sides in undulatory locomotion, probably each side having one going to the dorsal and one to the ventral longitudinal muscle sheets.

### *Neuromuscular relationships*

The fast responses in the longitudinal musculature associated with stimulation of nerve IV are of a type which appears characteristic of many invertebrate groups. Although the contractions show summation, the electrical responses decrease on every response after the first. This kind of response has been found in sipunculids (Prosser & Melton, 1954), holothuroids (Prosser, 1954), phoronids (Wilson & Bullock, 1958), polychaetes (Horridge, 1959), and cephalopods (Wilson, 1960). The rapid decline in some forms like *Neanthes* is probably not of great meaning for the intact animal since failure in other parts of the stimulus-response chain occurs in an all-or-none way at low frequencies. Thus the rapid innervation system probably never fires in a long repetitive sequence. This once-in-a-while rapid system is adequately suited to the startle response. At the same time it may be anticipatory to the more stable fast systems of the sorts found in arthropods and vertebrates. The apparent lack of refractory period in the longitudinal muscle suggests that all-or-none propagating action potentials do not occur, but that there are large junctional or local potentials which sum at any frequency. However, the

impossibility of working with a perfectly unfatigued preparation and the multiple nature of the system observed does not allow a confident statement that no refractory period exists for the individual fibre. The ability of the fast system to produce summing action potentials at high frequencies may depend on recruiting additional fatigued fibres with a second stimulus.

The muscles which make the rapid response as well as other muscles of *Neanthes* respond in another fashion also. Evidence is not available yet which will prove whether or not both responses occur in the same fibres. The slow innervation produces smaller but facilitating action potentials. The facilitation is not great and again this system could be a prelude to the highly facilitating systems of arthropods. The results with leeches show that they operate with a muscle control system similar to the slow system of polychaetes. Horridge & Roberts (1959), using a multifibre preparation, found facilitation of electrical response in earthworm muscle for the first two or three stimuli at 1–10/sec. Low-grade facilitating systems are apparently general among annelids. They have been found also in sipunculids (Prosser & Melton, 1954), echinoderms (Prosser, 1954), and cephalopods (Wilson, 1960).

### *Peripheral control*

The reflexes through the parapodial ganglia of *Neanthes* constitute a definite case of muscle control that is not routed through the central nervous system. However, this case represents a higher degree of organization than that associated with the concept of a peripheral nerve net. The characteristics of diffuseness and spread with repetition could not be found in *Neanthes*. It appears that each parapodial ganglion influences a definite region and that different ganglia are connected only by way of the central nervous system. A response to illumination of the posterior body wall of earthworms without nerve cord has been found, but it is weak and local (Prosser, 1946). This response is probably based on peripheral sensory-to-effector connexions similar to those in the parapodial ganglia, perhaps without involving a nerve net. Conduction in the leech body wall seems not to be synaptic, but to spread no farther than the extent of single nerve or muscle cells.

## SUMMARY

1. Nerve muscle preparations of the segmental nerves and associated muscles have been made using a nereid polychaete, *Neanthes brandti* (Malmgren).

2. Two kinds of response, differing in threshold and latency, were found. The 'fast' response is large at the first shock and (at frequencies above 1/sec.) decreases thereafter. The 'slow' response is small but facilitates with repetition at frequencies above 10/sec. Facilitation reaches a maximum after 3 or 4 shocks.

3. Isolated parapodia show several distinct reflex movements to mechanical and chemical stimuli. These must involve motor neurons in the parapodial ganglion.

4. Stimulation of the segmental nerves of the leech, *Hirudo*, evokes facilitating muscle potentials resembling in most details those of the 'slow' system in *Neanthes*.

5. The 'fast' and 'slow' responses are discussed in comparison with other invertebrate systems, especially those of arthropods. The 'slow' responses in annelids show less facilitation. The 'fast' responses of polychaetes fatigue quickly and are probably useful only in 'startle' responses.

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# NERVOUS CONTROL OF MOVEMENT IN CEPHALOPODS

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## INTRODUCTION

The innervation of cephalopod muscle is poorly understood, although it has been the subject of many studies. From the point of view of the physiologist who needs a workable nerve-muscle preparation it is especially disappointing that most of our knowledge concerns the tentacles and suckers. The nerves and muscles of the tentacles were described early in this century by Guerin (1908), and very extensively by Rossi & Graziadei more recently (1954, 1956, 1958). The latter authors describe peripheral cell bodies, anastomosing nerve nets, two size classes of fibre with questionable diplotomic branching, enlarged neural 'tubes' with fine branches, and endings which spiral around muscle cells. Sprenkel (1929) has described loop-like endings penetrating muscle cells. Martoja & May (1956) found both spiral and bouton endings in the same animals, *Octopus* and *Sepiola*, but in different muscles. They believe that the bouton terminals penetrate the muscle cell membranes and lie near the nucleus. Cells which are regarded as both cutaneous sensory and motor to muscle but independent of the rest of the nervous system were also found by these authors. Mikhailoff (1921) described peripheral nerve cells in cephalopod muscle which he thought were involved in both peripheral and central reflexes.

From methylene-blue studies Hofmann (1907*a*) made a reserved interpretation in favour of double innervation of the muscle fibres of the chromatophores. He found (1910) no diffuse peripheral nerve net associated with the chromatophores. The question of a peripheral nerve net in the mantle and fin muscle was examined by Hofmann (1907*b*). He suggested that anastomosing is probable in the subcutaneous plexus of the fin of *Sepia*, but that if a net is present it conducts with strong decrement. Cate (1929*a*) showed, on the one hand, the lack of dependence of the peristaltic waves on the continuity of a peripheral net in *Sepia* fin. On the other hand, he found that with local stimulation of the isolated fin excitation was conducted a certain distance.

The nerve supply of the mantle and fin, hence of the swimming and respiratory muscles, arises from the stellate ganglion (Fig. 1). The anatomy of the stellate ganglion is relatively well known. Reference will be made later to the works of Sereni & Young on *Octopus*. Graziadei (1959) has recently described the stellate ganglion of *Sepia*.

The aspects of control of movement of the cephalopod mantle treated here may be divided into two parts: the role of the stellate ganglion and neuromuscular trans-

mission. Concepts of the function of the stellate ganglion have had a controversial history. Von Uexküll (1894) viewed the ganglion as a motor relay centre without reflex function. The same is true of Baglioni (1905) who compared it to the ventral horn with lower motor neurons of vertebrates. However, he did not credit it with sensory feedback. A. Fröhlich & Loewi (1907) found spread of excitation over the mantle even after section of the mantle nerve, but not after removal of the ganglion. F. Fröhlich (1910*a*) stated that old or anoxic preparations lose this function quickly. More recently, the question as to whether the stellate ganglion is a reflex centre has been answered negatively by Bozler (1927), and positively by Cate (1929*b*) and Sereni & Young (1932). Early studies of transmission through the ganglion were made by F. Fröhlich (1910*b*) who demonstrated physiological synapses which facilitate and result in a long ganglionic delay.

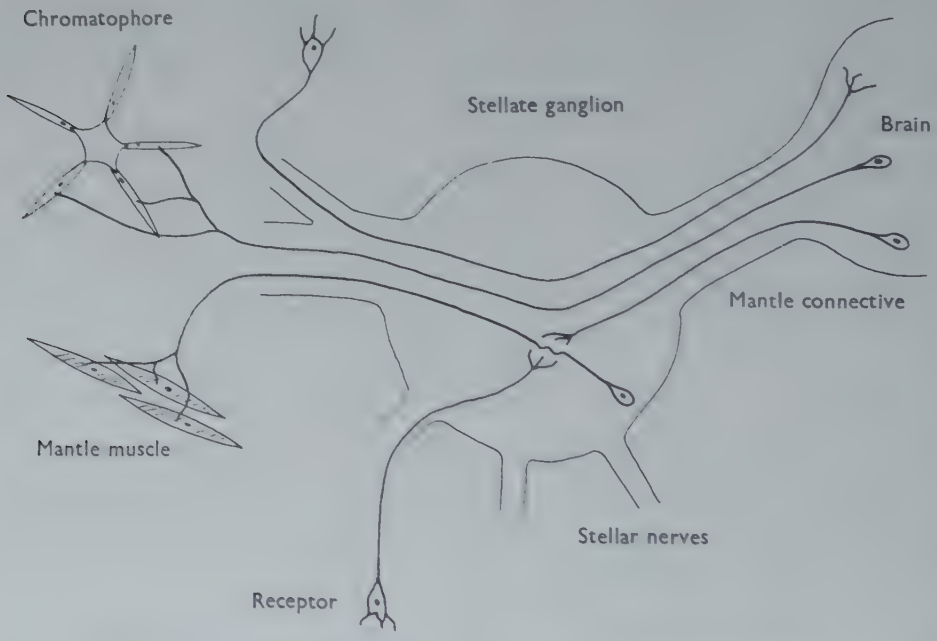


Fig. 1. Diagram of the stellate ganglion and associated nerves in *Octopus*.

A nerve-muscle preparation of the mantle and the stellar ganglion and nerves of octopuses was described by Burian (1908). F. Fröhlich used this both for the above-mentioned studies and to show some of the properties of the mantle muscle. He found summation of mechanical responses when stimulating the stellar nerves and facilitation when stimulating the mantle connective. Stimulation of the connective also resulted in inhibition of tonus in the mantle muscle.

As a result of the studies of Prosser & Young (1937) and Young (1938) the control of the squid mantle is better known than other aspects of cephalopod neuromuscular physiology. Each giant axon innervates a large area and produces non-

facilitating, virtually non-summating responses to each impulse. Many small fibres in the stellar nerves supply the same areas and produce contractions which vary with stimulus intensity and frequency.

## EXPERIMENTS ON OCTOPUSES

### *Material and Methods*

Two-spotted octopuses were obtained from southern California beaches. These were *Octopus bimaculatus* Verrill and *O. bimaculoides* Pickford & McConnaughey, two similar species separated most easily on the basis of egg size. No species differences are evident in the experimental results.

The nerve-muscle preparation was made by isolating one half of the mantle including one stellate ganglion and its stellar nerves. The mantle was pinned to a wax dish and flooded with sea water. Stimulation was either by means of paired silver wire electrodes holding a nerve out of the bath, or through a semi-micro tungsten needle plunged into the mantle or nerve. Electrical recording in the mantle was also through a tungsten needle probe. The tungsten electrodes were tapered by electrolytic etching in potassium nitrate (Hubel, 1957), and insulated to a point near the tips with insulex or glyptal. These electrodes were a few micra in diameter at the tip and thus suited to extracellular recording from a number of muscle cells but within a small area. For tension recording connexion was made to an RCA 5734 mechano-transducer tube. The recording device allowed only a very small amount of length change.

### *Anatomy*

#### *Description of the mantle muscle*

According to the description of Winkler & Ashley (1954) the mantle of *Octopus vulgaris* is composed of three layers, an outer longitudinal layer and two circular layers within. Burian (1908) lists, in addition, transverse or radial fibres in the mantle. In the species used in this study all four muscle groups exist. The outer longitudinal layer is thin, amounting to only about one-eighth of the whole thickness of the mantle. The two circular layers have the same direction, but are separated by a thin layer of connective tissue through which course the stellar nerves and their branches. All three layers are crossed by parallel bands of radially oriented fibres. The bands are only one to a few cells in thickness. Individual fibres in these bands cross the boundaries between the main layers.

#### *Muscle fibres and nerve fibres*

Using an octopus about average in size for this study (about 250 g.), estimates were made of the numbers of muscle fibres and nerve fibres. The muscle count was based on a relaxed mantle length of 6 cm. Muscle fibre size was observed after maceration in MaCallum's fluid (Guyer, 1953). In this, as in other cephalopods (Ballowitz, 1892; Plenk, 1933), the fibres are uninucleate and spirally striated. They have a length of 1–2 mm. and a maximum thickness of 8–9 $\mu$ and taper toward both ends. Allowing 50% for connective tissue and dividing the remaining mantle

muscle volume by muscle cell volume the mantle is shown to contain at least $2 \times 10^8$ fibres. The same mantle must have at least 1–2 million chromatophores.

The number of light-microscopically visible fibres in the stellar nerves was determined from sections prepared according to the technique of Sereni & Young (1932). The number in different nerves varied from several hundred to several thousand. No size classes were observed; instead a continuous spectrum of fibres having connective tissue sheath diameters of 3–15 $\mu$ was found. Electrical observations show a smoothly graded response having maximum velocity of 1·75 m/sec. at 24° C. Fröhlich (1910*c*) found a velocity of about 1 m/sec. at 15° C. The sum of the fibres of all the nerves was estimated at 20,000.

According to Sereni & Young (1932) only a few of the total nerve fibres in the mantle are sensory. In view of the fine control of colour pattern possessed by octopuses it may be postulated that a large proportion of the motor fibres innervate the chromatophores. As a conservative estimate, and without considering the possibility of multiple innervation, the motor unit in the mantle comprises tens of thousands of muscle fibres.

## *Physiology*

### *Responses of the mantle muscle to stimulation of the stellar nerves*

These observations may be divided into three parts: visual observations of gross movement, recordings of electrical events, and recordings of tension changes.

Most of the tests were made at room temperature, about 23° C. Although the natural environment is cooler, around 16° C., these animals can be maintained in apparent good health for months at room temperatures. The effect of temperature on neuromuscular control was checked once. At 15°, 22°, and 28° C. the responses were similar. Above 30° C., the responses were weaker, but not qualitatively different. The kind of response is not dependent upon temperature in this range.

Stimulation of the stellar nerves usually resulted in contractions which *reduced the area* of the mantle. With high intensity stimulation the contraction involved a sector of the mantle corresponding to the area innervated by the stellar nerve. At low intensities of stimulation discrete patches of contracting tissue responded in an all-or-none fashion. Repetition caused stronger contractions. Increases in intensity brought in new patches. Strips cut in the direction of the longitudinal or circular layers were stimulated directly and tested for differential activity in these two layers. Regardless of the direction of the strips stimulation resulted in some shortening. It was not possible to separate longitudinal and circular muscle contractions.

A different response was observed on a few preparations. Above a threshold value of stimulation, a *small patch of mantle thinned* conspicuously, causing the mantle to spread. The movement was not graded with intensity, but became greater with repetition. It is interpreted as due to selective activation of the radial muscle fibres. The existence of this kind of muscle activity is predictable from observations of the whole animal. Without the aid of peristaltic waves and with the aperture of the mantle open, the octopus may increase the area of its mantle and the volume of the mantle cavity.

The *electrical events* in the circular muscle layers of the mantle resulting from stimulation of the stellar nerves were of two kinds. At lowest intensity of stimulation the all-or-none patches could be mapped by means of the probing electrode. These patches had fairly sharp boundaries which did not change with repetition. In a specimen of 8 cm. mantle length the patches were 5–10 mm. in diameter. By changing the intensity and site of stimulation of the nerve different reactive units may be identified in the same preparation.

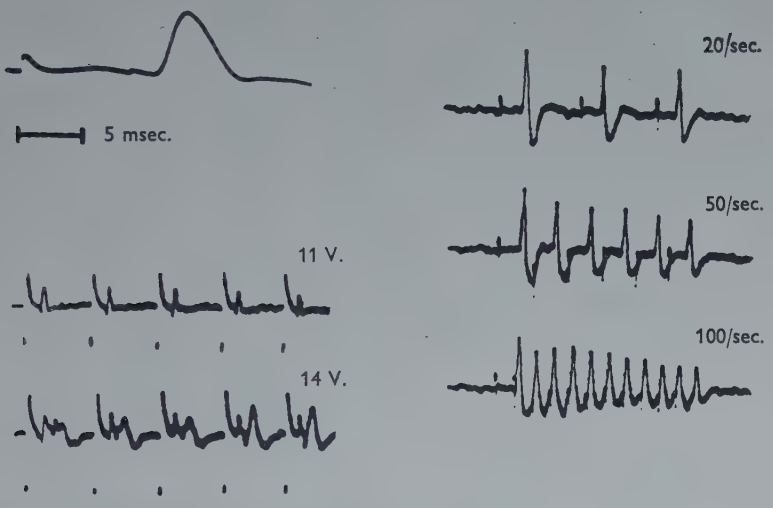


Fig. 2. All-or-none, threshold electrical responses in the octopus mantle. Left. Top: nerve potential and muscle action potential of fast response. Time marker, 5 msec. Bottom: response to varying voltage of stimulation of stellar nerve. At the higher voltage a slower, facilitating response occurs. Large initial deflexion is stimulus artifact. Frequency 20/sec. Right. Fast response at 20, 50, and 100/sec.

The electrical record in one all-or-none area is shown in Fig. 2. The response to a single shock was a muscle action potential having a duration of about 6 msec., the rising phase lasting about 2 msec. A small wave preceding the muscle action potential (Fig. 2) was interpreted as nervous activity. This would indicate a neuromuscular delay of nearly 2 msec. Repetition above 10/sec. resulted in slightly *decreasing action potentials*. The decrease was greater at greater frequencies, the response waning to one-half after about 10 shocks at 100/sec. At frequencies above 100/sec. action potentials did not follow long trains of stimuli in a one-to-one relationship. However, for pairs of stimuli the nerve-muscle preparation follows to very high frequencies. With stimulation of the stellar nerve the absolute refractory period for an all-or-none muscle unit was never greater than 1·6 msec. When the interval between stimuli was increased beyond the refractory period a second electrical response appeared suddenly, but about 1 msec. later than expected. It is suggested that this absolute refractory period is in the nerve and that the lateness of the second muscle response is due to slowing of nervous conduction. The second response fuses with the first, but the increment it adds becomes smaller with higher frequencies so that it never produces a peak higher than the first. The muscle

potential due to this fast innervation is maximal with the first excitation, but it has no absolute refractory period which outlasts the rising phase as is the case with spike potentials.

At higher intensities of stimulation complex action potentials appeared at a single electrode position (Fig. 2). In the simplest form these included two waves, one corresponding to that just described, and a later wave of longer duration and initially lower magnitude. This second wave increased rather than decreased with repetition, that is, showed facilitation, and at fusion frequency summated as well.

In most preparations, especially old ones in which the nerve had dried, some patches were found which *responded with facilitation*. These were presumably innervated by smaller nerve fibres which had outlived the larger low threshold ones. In a few cases these have also been found as unit-responding preparations, but usually they are compound. The area of innervation was much smaller than for the faster fibres, perhaps 2 to 3 mm. in diameter, but was not carefully mapped. The boundaries appeared to be diffuse rather than sharp. Different fibres gave different frequency-response relationships. Some showed facilitation at 5/sec. with a maximum at 30/sec.; others showed conspicuous augmentation only at 50/sec. or more. Maximum amplitude was reached after a few shocks, usually more than three but less than ten. The *minimum number of motor units* may be calculated assuming overlap of fast and slow motor units but no overlap of the same kind, and taking the average diameters of these units to be, respectively, 5 and 2 mm. in an octopus of average size for this study. This calculation indicates space for about 600 of the first kind and 4000 of the second. Together with the estimated total of 20,000 nerve fibres to the mantle, these figures suggest overlap of motor unit areas, but the level of error possible in these estimates does not permit a conclusive statement.

*Tension records* for the all-or-none fast type of response in the circular muscle began about 16 msec. after the start of electrical activity. The rising phase of the single twitch lasted about 0·16 sec. The relaxation was about 3 times as long. At frequencies between 2 and 4/sec. the twitches began to fuse, but the second was smaller than the first. Above 4/sec. summation occurred. Smooth tetanus occurred above 15 impulses/sec. The tetanus:twitch ratio was large, varying in different preparations between 20 and 100 to 1. Fig. 3 shows records of these responses.

Maximal stimulation of fresh preparations produced effects similar to those described for the single fast fibre. Paired shocks gave a maximal response about twice the amplitude of a single twitch. This maximum occurred at 30/sec. and was conspicuously reduced only below 10/sec. and above 60/sec. With maintained stimulation, tension increased rapidly at first and then more slowly for several seconds. The rate of increase in tension continued to rise with increased frequency to about 150/sec. The two separate phases of increase were the striking feature of this tetanus. Although this fast type of response shows a diminution rather than a facilitation of twitches, a post-tetanic potentiation occurred with maximal stimulation so that successive tetani were larger, within certain time limits (Fig. 3).

The mechanical response of the slow facilitating type was not within the range of the recording apparatus at threshold, but in old preparations, after the nerve had

partially dried, facilitated mechanical responses sometimes could be recorded with maximal stimulation. The response to two shocks was sometimes more than three times greater than to one. The facilitation began at 10–15/sec., fell off above 100/sec., and disappeared at approximately 160/sec.

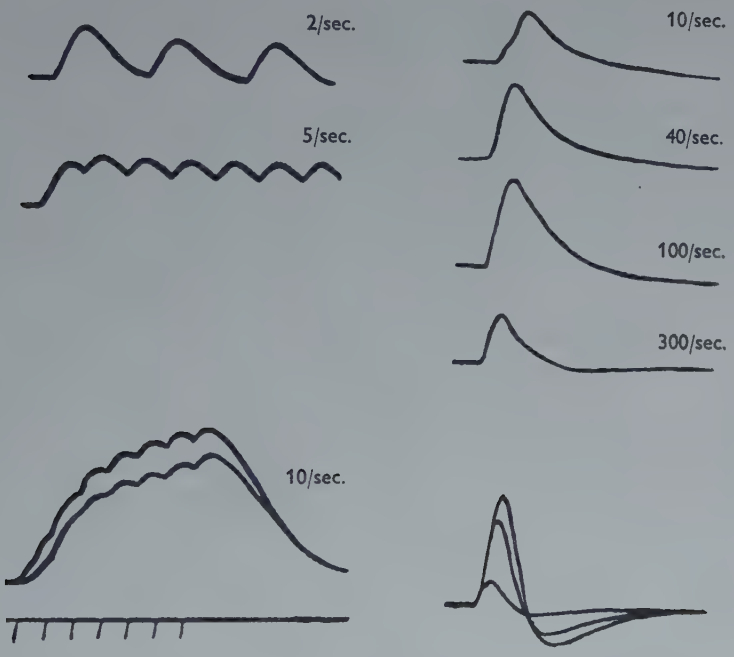


Fig. 3. Left. Tension in mantle with just above threshold stimulation of fast system in octopuses. Top: fast response in *Octopus* at 2 and 5/sec. Bottom: fast response in *Octopus* at 10/sec. repeated after 1 sec. Greater response is second series. Right. Mantle tension resulting from stimulation of many units of the slow systems of octopuses. Top: slow response of *Octopus* to pairs of shocks direct to the mantle at 10, 40, 100 and 300/sec. Bottom: slow response of *Octopus* at 30/sec. Superimposed records of 1, 2 and 3 shocks. Recorded with a.c. coupling to insure constant base-line.

*Response to direct stimulation of the mantle*

Small waves of electrical activity were propagated with a conduction velocity of less than 1 m/sec. away from stimulating electrodes within the mantle muscle. The maximum range of spread obtained was about 6 mm. The amplitude of the response was dependent upon frequency, but the degree of spread apparently was not. The mechanical response to this type of stimulation was always facilitated by repetition at near threshold values of stimulation. Thus there appeared a reversal of the thresholds of stimulation of the fast and slow systems between the stellar nerves and the periphery. The frequency response relationships of the slow system were more easily determined with the more peripheral stimulation because of this threshold reversal. The response to pairs of shocks was somewhat more than doubled over a single shock at 10/sec. The degree of facilitation increased to about five times

at 100/sec. At higher frequencies it waned. The facilitation for the third shock was less and at frequencies above 30/sec. the tension added by the third shock was no greater than the first (Fig. 3). At the lowest frequencies maximum facilitation was reached within four shocks.

*Effect of previous section of stellar nerves*

Mantles isolated from the central nervous system, either for a few minutes or many days, show considerable spontaneity in the form of weak local contractions. Mechanical stimulation produces stronger local contractions.

*No physiological evidence of degeneration* in the muscle innervation was found after section of the stellar nerves or removal of the stellate ganglion. Stimulation of the central ends of the stellar nerve stumps in octopuses kept at 15° C. for up to 34 days after removal of the ganglion gave normal non-facilitating electrical responses. In a 17-day-old preparation these responses fatigued more rapidly than in normal animals and were replaced by facilitating potentials like those of the slow system. Response to stimulation direct to the mantle was normal. In the oldest preparation, however, no facilitating responses were found; stimulation either at the nerve stump or more peripherally resulted in fast responses even after fatigue.

Sereni & Young (1932) found histological degeneration of the motor fibres within a few days after their section in octopuses kept at 25° C. Besides the possibility that such considerable difference in degeneration could be due to the 10° C. difference in temperature, several possible but untested explanations exist: (1) histological and physiological criteria of degeneration do not coincide and functional degeneration is slower; (2) central motor axons have nutritive contact with peripheral cell bodies; (3) only the slow fibres have such contact and hypersensitivity results in maximal electrical response to single shocks even though the fast fibres are inactive.

*Motor functions of the stellate ganglion*

*Synaptic relay of brain efferents.* Electrical stimulation in the mantle connective and recording in a stellar nerve gave the following results. At the lowest intensity producing any response a small electrical wave appeared in the stellar nerve. This was accompanied by a small contraction of the mantle. Repetition was followed by increasing potentials indicating synaptic integrative processes in the ganglion. At higher intensity an earlier wave appeared, associated with expansion of the chromatophores. It did not show frequency augmentation. The higher intensity necessary for stimulation of these fibres, indicating that they are smaller than the former, together with the shorter latency and lack of frequency grading, suggest that they pass without synapse through the ganglion. Long-continued stimulation resulted in much earlier fatigue of the mantle motor response than the chromatophore response. After this fatigue, stimulation distal to the ganglion resulted in contractions again, but not so in the case of fatigue of the chromatophore response. These results agree with the anatomical studies of Sereni & Young (1932). Excitatory motor fibres to the chromatophores travel from the brain directly to the

periphery, the mantle motor fibres synapse in the stellate ganglion where facilitation to repetition and perhaps after-discharge occurs.

Stimulation of the mantle connective resulted in a complex wave of electrical activity in the mantle muscle. At frequencies around 1/sec. the latency decreased slightly and the magnitude increased with repetition. The resulting facilitation lasted several seconds. Contractions in the mantle due to stimulation of the mantle connective showed a larger degree of facilitation than stimulation more peripherally.

*Reflex function from peripheral stimulation*

Stimulation of the proximal stump of the largest stellar nerve also showed greater facilitation than more peripheral stimulation. Such afferent stimulation caused mild contractions over much of the same half of the mantle even when the mantle connective was cut. Stimulation of the mantle connective and the proximal stump of a stellar nerve simultaneously, or nearly so, resulted in a summated response.

It is unlikely that the contribution from the stellar nerve was via an axonal reflex or other through-conducting pathway. Its latency was longer than would be predicted by the increase in conduction distance across the ganglion as compared to efferent stimulation of a stellar nerve. The duration of the response to a single stimulus was much longer than that resulting from stimulation not across the ganglion. It seemed to fatigue more rapidly than the simple nerve muscle preparation. In the partially fatigued preparation there was no recordable response to one or a few shocks, or to frequencies as low as one or two per second, but large responses followed long and high-frequency bursts. The response did not have a simple relationship to the stimulus, probably due to the interaction of antagonistic muscles. Often the relaxation following cessation of stimulation was extraordinarily rapid and showed considerable and long-lasting overshoot. This is reminiscent of the inhibition of tonus reported by Fröhlich (1910*d*), but might also be explained as belated or more enduring reaction of antagonistic fibres. The possibility that these reflexes are mediated by an artificial synapse at the cut end of the mantle connective is slight since the sea-water bath would provide a short circuit.

Degeneration studies done by Sereni & Young (1932) demonstrate that most of the fibres present in the stellar nerves and visible in the light microscope are motor. Exact counts are not given, but the authors state that only a very small percentage of the fibres are sensory. The modalities of the fibres which cause the reflex activity are unknown. There was no evidence in reflexes of the negative feedback which might be expected in the case of stretch receptors.

## EXPERIMENTS ON SQUIDS

### *Squid preparation*

*Loligo pealeii* (Lesueur) were studied at Woods Hole and *L. opalescens* Berry in Los Angeles. The preparation and recording techniques were the same as for octopuses. The effect of small fibre stimulation was determined either by using the fin nerve or by damaging the giant fibre in a stellar nerve.

*Response of the mantle to giant fibre stimulation*

Mechanical responses were obtained which agree in every respect with those of Prosser & Young (1937). At threshold, all-or-none muscle twitches occurred to each stimulus. These did not facilitate nor even sum noticeably, but did fuse at frequencies above about 5–10/sec., giving a small tetanus:twitch ratio (*c.* 1).

Electrical recording produced additional information about the giant fibre system. Muscle action potentials lasting about 10 msec. follow giant fibre stimulation. These *decreased in amplitude* with repetition over 20/sec. Pairs at intervals shorter than 10 msec. fused, at least in partially fatigued specimens, but the second response was always lower than the first. At no frequency could uphill *treppe* be produced.

After fatigue to 5 or 10 % of its original fresh level the giant muscle action potential takes on the characteristics of a slow innervation system. In the fatigued condition successive responses grew slightly at repetition rates above 10/sec. These responses were associated with a low threshold all-or-none unit in the stellar nerve which could not be other than the giant fibre.

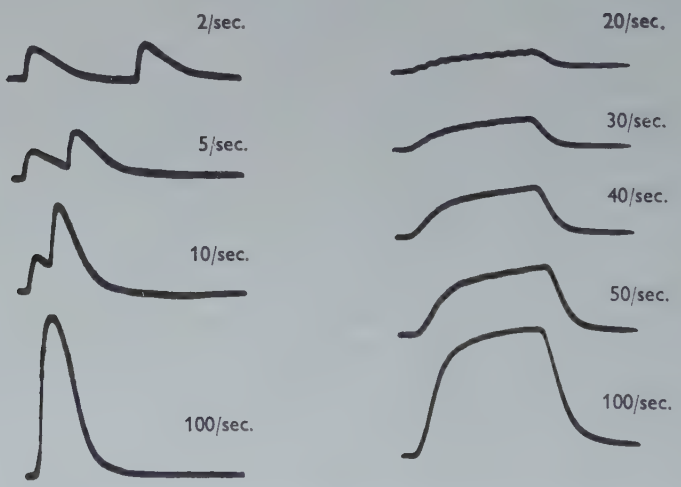


Fig. 4. Tension in the squid mantle due to stimulation of many small fibres of a stellar nerve. Left. Slow response at 2, 5, 10 and 100/sec. near maximum paired stimulation of stellar nerve with damaged giant fibre. Right. Response at 20, 30, 40, 50 and 100/sec. maintained for $\frac{1}{2}$ sec.

*Responses to small fibre stimulation*

*Facilitating potentials* with higher threshold and longer latency could be produced by stimulation of the same nerve in which the giant axon response was fatigued. Similar facilitating muscle-action potentials occurred when fin nerve or stellar nerve with damaged giant axon was stimulated. This facilitating response corresponds to the frequency-graded responses found by Young (1938). The degree of facilitation was larger than for fatigued giant axon stimulation. Facilitation followed only the first few shocks at frequencies greater than 4/sec. Fusion began at 100/sec.

*Mechanical records* during small nerve fibre stimulation were similar to the slow responses of octopuses (Fig. 4). Facilitation occurred above 5/sec. Response to pairs was maximal at 100/sec. and decreased slightly to above 300/sec. when it fell off abruptly, presumably due to failure of nerve conduction. Maximum facilitation was reached after 4 or 5 shocks at low frequency (Fig. 5) and in fewer shocks at higher frequencies.

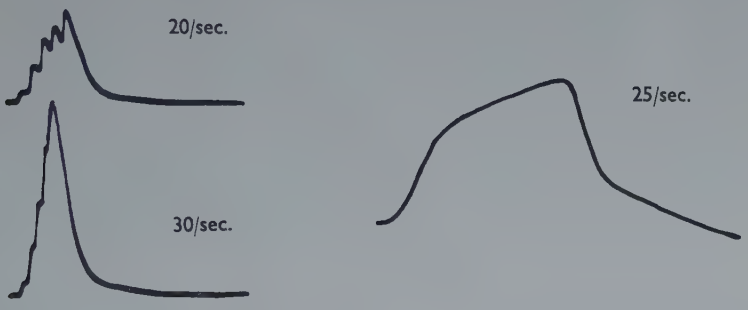


Fig. 5. Tension in the squid mantle due to stimulation of many small fibres of a stellar nerve. Left. Response to 5 shocks at 20 and 30/sec. Right. Response at 25/sec. for ½ sec.

Long trains of stimuli resulted in tetanic contractions with smooth fusion of responses above 30/sec. (Fig. 4). The tetanus : twitch ratio at 30/sec. was about 100 : 1. The strength of the contraction increased disproportionately with increase in frequency at least to 100/sec. As in *Octopus* mantle, two phases of the tetanus could be seen (Fig. 5). A rapid initial rise was followed by a slower and long continued increase in tension. Relaxation was likewise rapid at first and then slower.

Stimulation of the small fibres of the stellar nerves *never resulted in fast type responses*, although wide ranges of intensity and duration of stimulus were employed.

## DISCUSSION

### *Organization of the motor apparatus*

The breathing and swimming movements of octopuses and squids involve alternate increases and decreases in the mantle volume. The muscular movements which effect these changes consist in reciprocal activity between antagonistic fibres in the mantle. Since the mantle cavity is normally open, the necessary changes in shape cannot result from the activity of circular and longitudinal muscles operating alone against a turgor skeleton, but probably are due also to radial musculature making use of some rigidity of the mantle itself. Radial fibres have been described for squids (Young, 1938) and were found for octopuses by Burian (1908). In octopuses, nerve fibres were found which selectively activated muscle fibres causing thinning and expansion of the mantle. These muscle fibres are undoubtedly the radial ones. These radial fibres oppose both the circular and longitudinal fibres which usually appear to act together. In this study no means could be found to separate the action of the last two layers and it is suspected that they have overlapping innervation. The suggestion has been made by Hofmann (1907*b*) that the layers of the fin of *Sepia*

are nervously linked. However, Fröhlich (1910*d*) was able to elicit separate contractions of the longitudinal and circular layers of *Octopus macropus* by stimulation of the intact animal. It is not impossible that the two systems of innervation, facilitating and non-facilitating, differ in this respect, with perhaps one system activating the layers separately and the other activating them together. The several histological studies do not elucidate these matters, nor do they exclude or support a double innervation of muscle fibres.

Normal use of the mantle depends on its *connexion to the central nervous system.* Since the two halves of the mantle normally act together, but are not dependent upon each other (cutting one mantle connective and therefore the commissure between the ganglia does not affect the other side), control of respiration and swimming is probably from higher centres than the stellate ganglion. However, it is clear that the stellate ganglion is involved in integrative processes affecting the activity of the mantle muscle. The older anatomical and physiological findings (Fröhlich, 1910*b*; Bullock, 1952), as well as the present evidence, demonstrate the presence of synapses along the motor pathway to the mantle. These synapses are not one-to-one but are facilitated to shorter latency and larger discharge, and probably after-discharge, by repetition of presynaptic stimulation. This activity of the ganglion may result in a smooth grading of a simple central command analogous to that made possible by the high degree of summation and facilitation found more peripherally in arthropods. The evidence is strongly in favour of a reflex function in the ganglion as well. It is not possible to say yet whether this is primarily proprioceptive and feedback in nature, or whether it is primarily exteroceptive and therefore relatively independent of more central control. It seems probable that in the intact animal the command for movement of the mantle operates through the brain and that the stellate ganglion mainly brings about a smoothing of control.

*Gradual contractions superimposed on faster ones* were found in the case of tetanic stimulation. These resulted in sloping tetanic plateaux following stimulation of the small fibres of squid or octopuses. They do not occur with pure giant fibre stimulation. Tonic contractions were never observed by themselves and it is unknown whether they can be elicited centrally or whether they are a local phenomenon elicited by faster contraction. No evidence was found of fibres in the stellar nerves which inhibit peripheral tonus. However, the evidence of Bozler (1928) on the chromatophores and the findings of Fröhlich lead one to expect such inhibitory mechanisms in the mantle. The extraordinarily rapid relaxations, sometimes with overshoot, which often followed preganglionic stimulation, resemble Fröhlich's results, but are here interpreted as due to interactions of antagonistic muscles.

The *reversal of threshold* between the fast and slow innervation systems dependent on whether stimulation is in the proximal part of the nerves or in the muscles takes place in *Octopus* but not in *Loligo*. The giant fibres, the only ones which mediate a fast response in squids, apparently remain relatively large even within a small distance of their terminations. In the octopus mantle the slow fibres may be associated with some larger structures such as peripheral cells or the neural tubes described by Rossi & Graziadei (1958). Some nervous structure must account for

the slow type of excitation which takes place when the mantle is stimulated directly. The constant limit to which such excitation will spread suggests that the stimulus does not directly affect the muscle cells and then conduct from muscle fibre to muscle fibre.

The present results agree with those of Hofmann and Cate on the presence of *peripheral conduction* of excitation. All concur that spread of contraction with repeated stimulation takes place over short distances. Limited spread cannot, by itself, be taken as proof that no nerve net is functioning (Horridge, 1957), but other evidence does suggest this. The actual distances found in this study are similar to the diameter of the fields of influence of single units in the stellar nerves. The conduction velocity for the electrical wave, nearly 1 m./sec., is high for a diffuse network. The simplest interpretation is that no physiological nerve net exists, that local reactions spread only along the branches of a single fibre, and that the histologically evident plexus does not represent anastomosing terminal branches of many units.

No physiological evidence has been advanced on the peripheral neurons supposed by several anatomists to be motor neurons.

### *Neuromuscular relationships*

The large number of muscle fibres innervated by a single nerve fibre in cephalopods does not raise the same question that it does in the case of the arthropods. In the case of arthropods a single axon or a few axons must control the movement of whole muscles and gradation of response cannot be like that of vertebrate motor units. In cephalopods, while the ratio of muscle fibres to nerves is even higher, the number of nerve fibres to a large muscle is considerable. On the evidence up to this study, any of the following means of grading tension was possible: (*a*) strictly all-or-none activation of the entire field of innervation of individual axons such as is typically found in vertebrates with grading by recruitment; (*b*) all-or-none activation of each muscle fibre but with different amounts of repetition required to produce this activation in different fibres, as is found in curarized vertebrate muscle (Adrian & Lucas, 1912); (*c*) graded activity in single muscle fibres dependent on local phenomena which increase or spread with repetition, as is typical of arthropods; and (*d*) peripheral inhibition superimposed on any of the above.

*Two kinds of response* are found in octopuses and squids. The slower, *facilitating responses* are so similar in the two forms, both in degree and time-course of facilitation, that it seems probable the two systems are homologous. In these animals the degree of facilitation is not large. Minimal contraction does not require two stimuli as in some coelenterates (Pantin, 1935) nor does the facilitation increase beyond the first few stimuli as it does in arthropods (Pantin, 1934). The magnitude of neuromuscular facilitation in cephalopods seems to be more like that of annelids (Wilson, 1960) than other groups which have been studied.

The *non-facilitating reaction* of octopuses and the giant response of squids are similar in many ways. The motor unit area is, however, of the order of 100 times larger in squids. In each case the electrical response to the first stimulus is as large

as, or larger than, that to later stimuli. The mechanical response may show summation as in *Octopus*, but not facilitation, or may be almost maximal to a single impulse in *Loligo*. In both *Octopus* and *Loligo* stimulation of the fast systems at relatively low frequencies (10/sec.) results in diminishing action potentials, but this characteristic is not as marked as in polychaetes. Such diminishing potentials in whole muscle might be due either to gross failure of some number of units to respond after a few repetitions or to smaller electrical response by each unit. The apparent lack of refractory period during the muscle potential argues against propagating spikes, and it appears unlikely that failure in single fibres is all-or-none; these considerations support the second alternative. The anatomical evidence available includes descriptions of broad or multiterminal neuromuscular endings and allows the notion that local potentials could excite whole muscle cells. Another promising case for local response in cephalopod muscle is that of the denervated chromatophore muscle. This preparation was found by Bozler (1928) to respond in proportion to stimulus intensity, and although he was unwilling to accept this as a property of a pure single unit, this seems at least as likely as an alternative.

The two kinds of muscle excitation in cephalopods bear many similarities to those of the snail. Ramsay (1940) found two kinds of contraction in the buccal retractor. Electrical records showed an early potential which declined with repetition followed by a later augmenting potential. The first response was not maximal to the first stimulus as it is in cephalopods, and did have an absolute refractory period. Physiological evidence of double excitatory systems have been found in the clam (Pumphrey, 1938) and both tonic and phasic excitatory innervation as well as tonus inhibitory innervation are indicated in *Mytilus* (Hoyle & Lowy, 1956). Schmandt & Sleator (1955) found that action potentials in *Mytilus* muscle conducted with decrement in individual fibres, facilitated with repetition, and had little or no absolute refractory period. They found post-tetanic potentiation lasting minutes. The neuromuscular mechanisms found in cephalopods are at least represented, and are probably general, in the other mollusc groups.

Probably both the fast and slow systems in the cephalopods studied, and in *Neanthes* (Wilson, 1960), operate by means of *local potentials* and grading in individual fibres rather than spike potentials and recruitment. The fast system in cephalopods at least normally operates with a maximum electrical response to the first nerve impulse.

## SUMMARY

1. Nerve muscle preparations have been made of the mantle and stellar nerves of octopuses and squids.

2. Two motor innervation systems have been found in each. Both have been observed as unit preparations. The possibility of double innervation of the same muscle cells exists but has not been directly checked.

3. The fast innervations produce electrical responses which are maximal to the first stimulus and which have little or no absolute refractory period. They appear to be local rather than spike potentials. Fatigue is very rapid. The mechanical response sums in *Octopus*, but not in *Loligo*.

4. The slow innervations produce electrical and mechanical responses which facilitate with repetition. The fast system of *Loligo* does likewise after fatigue to a low level of response.

5. No evidence was found for a functional nerve net in the mantle.

6. Organizational features of the stellate ganglion have been identified physiologically in *Octopus*. The ganglion acts both as an integrating motor centre and as a reflex centre.

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# THE PHYSIOLOGY OF CONTRACTILE VACUOLES

## XI. EFFECTS OF HEAVY WATER ON THE WATER BALANCE OF A SUCTORIAN

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### INTRODUCTION

It is widely assumed that $D_2O$ may be used as a tracer for studying the water exchanges in living materials, provided that the damage sometimes associated with high concentrations is avoided. This conclusion is supported by the work of Lucké & Harvey (1935) for *Arbacia* eggs and of Løvtrup & Pigoń (1951) for the giant amoeba *Pelomyxa carolinensis* (*'Chaos chaos'*), but not by that of Parpart (1935) and Brooks (1935) on mammalian erythrocytes. In view of the growing use of heavy water in osmotic and diffusion studies more information about its permeation is desirable.

The osmotic passage of water through freshwater Protozoa, as shown by the contractile vacuole, offers an exceptionally favourable opportunity for examining the permeation of heavy water. Previous work on Protozoa (Gaw, 1936) is not satisfactory for the present purpose.

### METHODS

The suctorian *Discophrya collini* (Root) (=*D. pyriformis* Guilcher) was used in nearly all experiments. It was grown on silk threads in Chalkley's solution for some experiments of series 1 and in Bristol tap water for all other experiments. *Colpidium* sp. was given as food, and *Discophrya* was used 5–10 days after the culture had been fed. For two experiments the peritrich *Carchesium aselli* was obtained on the legs of the freshwater isopod *Asellus aquaticus*.

A few of the silk threads carrying *Discophrya* were laid on a slide under a short length of cotton thread which served to anchor them, and were mounted under a cover-glass. The preparation was irrigated very frequently with a hand pipette, helped by filter-paper leads. A change of solution could be effected very quickly. For each experiment a single individual with clear protoplasm and one contractile vacuole was chosen for observation.

For experiments of series 1 the *Discophrya* was irrigated: (i) with Chalkley's solution made up with ordinary distilled water ($H_2O$); (ii) with Chalkley's solution made up with a known mixture of $H_2O$ and $D_2O$; (iii) the same as for (i).

For experiments of series 2 the sequence of treatments was: (i) a known mixture of Bristol tap water and ordinary distilled water; (ii) as (i) but with the distilled water replaced by $D_2O$; (iii) as (i).

Observations were only started after a period of irrigation with the solution to be used for the first period of the experiment. This preliminary irrigation lasted for about 2 hr. for series 1 and 15–60 min. (normally 30 min.) for series 2. The initial rate of output showed no drift. Air temperature near the microscope was between 17° and 24° C. for series 2, and variations did not normally exceed $\pm 0{\cdot}5$° C. in any one experiment.

The heavy water used in these experiments was supplied by A.E.R.E. and was of Norwegian origin. For all but preliminary tests it was re-distilled; this made no difference to the results. Its purity was confirmed by a measurement of conductivity made for us by Dr A. Couper and by an examination by mass spectrograph carried out for us by Dr W. J. Dunning.

## RESULTS

The results in the two series of experiments were alike. The results for series 2, summarized in Table 1, are further supported by some additional experiments with observations on body volume only. One experiment of series 2 is depicted graphically in Fig. 1.

On transfer to media made up with 5% or 10% $D_2O$ there was a rapid but transient fall in rate of vacuolar output. In 25% $D_2O$ and over there was at first a distinct wrinkling of the body which was clearly shown by measurement to be due to a volume shrinkage and not to a surface expansion. This temporary shrinkage took place within half a minute and was very considerable in the undiluted $D_2O$ (99·7%). While the body was noticeably shrunk, the activity of the contractile vacuole was suspended. With recovery of the normal body volume the contractile vacuole resumed activity and was soon evacuating water at the normal average rate, although there were periodic fluctuations in a number of cases. In many experiments the distal ends of the tentacles contracted spirally as the body shrank. Later they re-extended, but appeared swollen and never straightened fully in the undiluted $D_2O$.

On return to the original medium there was no measurable change in the dimensions of the body, but if a rather high concentration of $D_2O$ had been used there was at first an appearance of greater roundness and turgidity, which later disappeared. Sometimes some of the tentacles dropped off. Immediately after transfer the rate of vacuolar output increased, but later it fell to about the usual level. In general the higher had been the concentration of $D_2O$, the greater was the temporary maximum.

Owing to the much higher frequency of systole it was difficult to follow the changes in *Carchesium aselli* subjected to $D_2O$–$H_2O$ mixtures. A brief fall in the rate of output was found on transfer to 25% $D_2O$ in tap water, and a brief rise on return to the corresponding distilled $H_2O$ and tap-water mixture. With undiluted 99·7% $D_2O$ there was a severe temporary shrinkage of the body and a temporary

Table 1. *Effects of water containing* $^2H$ *on* Discophrya collini

| Exp. no. (and total no. of systoles) | Percentage of heavy water in mixture | Duration of treatment (min.) | Rate of vacuolar output: average (and range) in $\mu^3$/sec. and notes† | No. of systoles contributing to data in previous column | Body: appearance and volume |
|---|---|---|---|---|---|
| 240359 (60 systoles) | 0 | 26 | 0·76 (0·6–1·0) | 12 | Normal |
| | 10 | 43 | Fell to 0·39; normal in 9 min. | | No change seen |
| | | | 0·80* (0·6–1·0)* | 10* | |
| | 0 | 48 | Rose to 2·0; normal in 5 min. | | No change seen |
| | | | 0·83* (0·7–1·0) | 13* | |
| 210559 (60 systoles) | 0 | 17 | 1·34 (1·2–1·5) | 9 | Normal |
| | 10 | 51 | Fell to 0·55; normal in 5 min. | | |
| | | | 1·49* (1·0–2·2)* | 10* | No change seen |
| | 0 | 46 | Rose to 3·83; normal in 6 min. | | |
| | | | 1·30* (1·1–1·9)* | 11* | No change seen |
| 210359 (64 systoles) | 0 | 13 | 1·33 (1·0–1·8) | 6 | Normal |
| | 25 | 37 | Fell to 0·28; normal in 16 min. | | No change seen |
| | | | 1·53* (1·2–2·8)* | 13* | |
| | 0 | 25 | Rose to 6·0; normal in 7 min. | | No change seen |
| | 25 | 18 | Fell to 0·42; normal in 7 min. | | Slight temporary kink |
| | 0 | 16 | Rose to 4·3; normal in 5 min. | | Normal |
| 220459 (110 systoles) | 0 | 19 | 0·80 (0·7–1·0) | 17 | Normal |
| | 25 | 33 | Fell to zero; normal in 12 min. | | Slight temporary shrinkage |
| | | | 0·71* (0·5–1·0)* | 20* | |
| | 0 | 65 | Rose to 3·76; normal in 3 min. | | Normal |
| | | | 1·24* (0·9–1·7) | 17* | |
| 230359 (87 systoles) | 0 | 24 | 1·24 (1·0–1·7) | 7 | Normal |
| | 50 | 45 | Fell to zero; active again in 25 min.; normal in 46 min. | | A temporary kink; normal again after 25 min. |
| | | | 1·32* (0·8–1·9)* | 10* | |
| | 0 | 78 | Max. 9–11 in 2 min.; normal in 30 min. | | A very slight shrinkage as max. output subsided to normal; later normal |
| | | | 1·29* (1·2–1·5)* | 7* | |

† All times are given from the beginning of a treatment.
'Normal' implies approximately within the normal range of variation as found in the first period.
* For the last 20 min. of the treatment.

Table 1 (*cont.*)

| Exp. no. (and total no. of systoles) | Percentage of heavy water in mixture | Duration of treatment (min.) | Rate of vacuolar output: average (and range) in $\mu^3$/sec. and notes† | No. of systoles contributing to data in previous column | Body: appearance and volume |
|---|---|---|---|---|---|
| 250359 | 0 | 27 | 1·88 (1·5–2·4) | 19 | Normal |
| (103 systoles) | 50 | 47 | Zero for first 20 min.; normal in 24 min. | | Slight wrinkling first 15 min., then recovered |
| | | | 2·08* (1·6–3·4) | 21* | |
| | 0 | 44 | Max. 13–27 in 2–3 min. normal in 10 min. | | Very full at first; slight wrinkling (8 min.) then normal (20 min.) |
| | | | 2·01* (1·7–3·0)* | 17* | |
| 260359 | 0 | 21 | 1·75 (1·2–2·5) | 16 | Normal |
| (109 systoles) | 99·7 | 64 | Zero for first 20 min.; normal in 23 min. | | Severe temporary shrinkage; normal after 20 min. |
| | | | 2·11* (1·6–2·8)* | 17* | |
| | 0 | 42 | Max. 14·7 in 3 min.; normal in 20 min. | | Normal |
| | | | 1·72* (1·4–2·3)* | 22* | |
| 230459 | 0 | 13 | 1·68 (1·1–2·2) | 20 | Normal |
| (198 systoles) | 99·7 | 56 | Zero for first 15 min.; normal in 26 min. | | Severe shrinkage; normal after 25 min. |
| | | | 1·72* (0·9–4·4)* | 32* | |
| | 0 | 44 | Max. 8–12 in 2–3 min.; normal in 15 min. | | Normal |
| | | | 2·10* (1·6–2·7)* | 52* | |
| 140959 | 0 | 23 | 2·00 (1·6–2·5) | 15 | Normal |
| (126 systoles) | 99·7 | 76 | Zero for first 15 min.; rose to 8–10 at 23–24 min., then fell rapidly to normal range | | Severe shrinkage, followed by swelling until bloated; then normal |
| | | | 1·96* (1·5–3·0)* | 19 | |
| | 0 | 34 | Rose to 25–27 within 1 min., then fell to normal range | | Normal |
| | | | 2·15* (1·2–2·9)* | 34 | |
| 150959 | 0 | 19 | 1·41 (1·0–2·0) | 23 | Normal |
| (145 systoles) | 99·7 | 68 | Zero for first 18 min.; rose to 2–5 at 24–27 min., then fell rapidly to normal range | | Severe shrinkage, normal after 18 min. |
| | | | 1·26* (0·4–2·0)* | 16 | |
| | 0 | 37 | Rose to 10–13 in 1 min., then fell to normal range | | |
| | | | 1·13* (0·5–1·6)* | 35 | Normal |

* See p. 75 for footnotes.

stoppage of the contractile vacuole. Normal size and approximately normal rate of output were later restored, but there was some irregularity of the contractile vacuole and a noticeable reluctance of contributory vacuoles to fuse. On return to ordinary water there was a great temporary increase in rate of vacuolar output.

## DISCUSSION

The osmotic effects of water containing deuterium on *Discophrya* resemble those of a solution of ethylene glycol (Kitching, 1951), and are to be explained in a similar way. It appears that $D_2O$ (or DHO) penetrates more slowly than $H_2O$, so

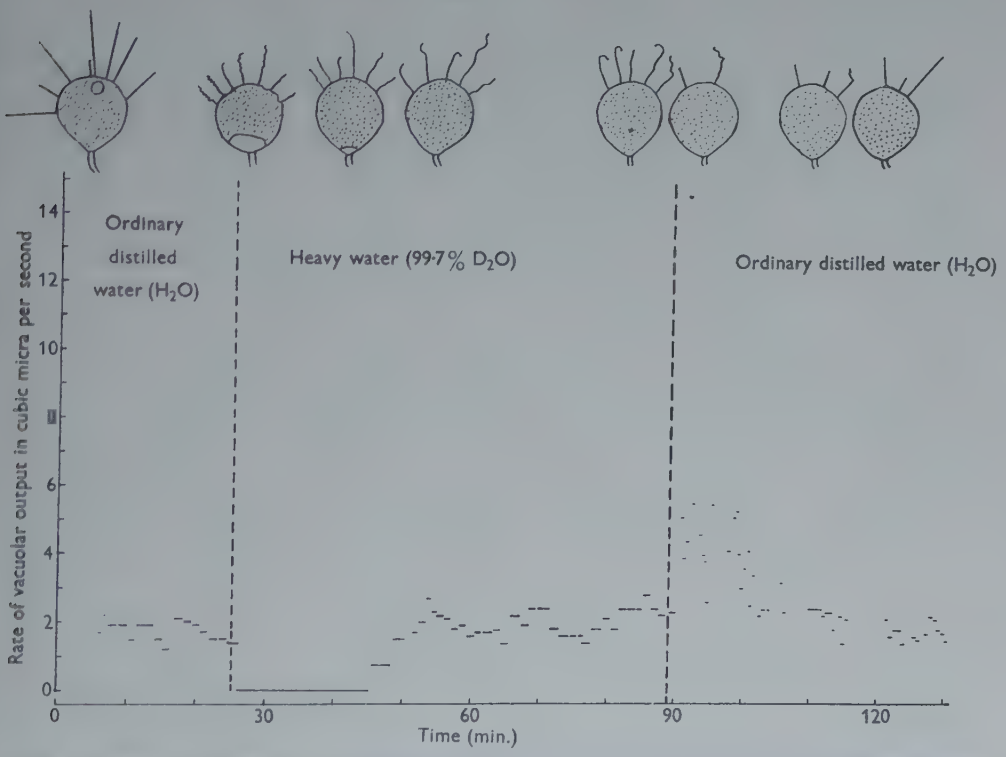


Fig. 1. Effect of $D_2O$ (99·7 %) on the rate of output of the contractile vacuole of *Discophrya collini*. Note the single high point at 14·7 $\mu^3$/sec. shortly after return to ordinary water. Changes in general appearance are shown above. The unstippled area adjacent to the stalk, which appears on transfer to heavy water, is a region of the body which has collapsed and is very thin.

that the normal osmotic influx into the organism is temporarily reduced (or reversed) when the organism is transferred to $D_2O$, and augmented when it is returned to $H_2O$. The slow recovery of a normal body volume while the organism is still in $D_2O$ may be ascribed to the osmotic uptake of water due to internal solutes. It culminates in the re-establishment of an approximately normal vacuolar output, and no doubt in an equality of isotopic composition of the water inside and outside the animal.

There is clear indication in some experiments that restoration of output is delayed until the body has swollen to a size slightly greater than normal, and in these cases when the contractile vacuole resumes activity it does so at an abnormally high rate of output. The marked fluctuations in rate of output which occur in $D_2O$ are probably of the same general nature, and may be ascribed to a lag in response of the controlling mechanism, which appeared to be less sensitive in $D_2O$, so that the oscillations inevitably associated with self-regulation are exaggerated.

A slower rate of haemolysis of erythrocytes in solutions made up with heavy water was attributed by Parpart (1935) in part to a slower rate of diffusion and by Brooks (1935) largely to a supposedly lower fugacity; other properties were also invoked. It is difficult from our observations to distinguish between explanations based on a kinetic property and those based on an equilibrium property of heavy water, although it appeared that a kinetic approach might be tested rather approximately.

The basic assumptions for a kinetic interpretation of our results are given in the Appendix, together with the conclusions worked out for us by Dr M. H. Rogers of the Department of Mathematics, University of Bristol. It is possible to test two features of our results, the extent of shrinkage, and its rate (very approximately), although both are obscured by the wrinkling of the apparently inelastic body surface. The working out is greatly complicated by the occurrence of the reaction $H_2O + D_2O \rightleftharpoons 2HDO$, which we assume to occur instantaneously. At the temperature of our experiments $[HDO]^2\,[H_2O][D_2O] = 3{\cdot}25$ (Farkas, 1935).

If the formation of DHO is ignored, $V_\infty / V_0 = D_{D_2O} / D_{H_2O}$, where $V_\infty$ = ultimate volume (theoretically) and is taken as the minimum volume (attained before osmotic swelling becomes effective), $V_0$ = original volume, and $D$ = Fick's diffusion constant (for water passing through the surface membrane of the organism). With formation of DHO the minimum volume will be very slightly smaller (see Appendix). Estimation of minimum volume from our results can only be made either very crudely or indirectly. One gets the impression of a rather considerable shrinkage. Comparisons with a tough rubber bladder filled to varying degrees with water have suggested a minimum volume not greater than 80–90 % of normal and possibly less. Estimation by measurement in two favourable cases in which the body surfaces became locally inverted in the form of a spherical segment gave 88 and 92 %. Indirect estimates were obtained by calculating the amount of water which must have entered by osmosis from the beginning of the treatment with $D_2O$ until the contractile vacuole resumed its activity; for this purpose the rate of entry was taken as (approximately) equal to the rate of vacuolar output after a steady state had been achieved. Estimates of minimum volume by this method for the last four experiments summarized in Table 1 are 86, 81, 91 and 91 %.

The rather rapid shrinkage which we have observed may be compared approximately with the rates to be expected from possible values of $D_{H_2O}$ and $D_{D_2O}$. If we neglect bulk flow or pore effects, considered by Prescott & Zeuthen (1953) to be small in the case of this amoeba, $D_{H_2O}$ may be calculated from the osmotic filtration constant ($F$) by the method of Løvtrup and Pigoń (1951); $F$ is estimated from the rate of vacuolar output ($1{\cdot}75\ \mu^3$/sec. for Exp. 260359), from the surface area

(3700 $\mu^2$ for this experiment), and from the difference of osmotic pressure across the membrane (estimated indirectly by Kitching (1951) as about that of a 0·05 M solution of non-electrolyte): $F = 0{\cdot}023\ \mu^3/\mu^2$/atm./min. and $D_{H_2O} = 0{\cdot}5\ \mu$/sec. If we suppose that $D_{D_2O}/D_{H_2O} = 0{\cdot}9$, which is a maximum figure on the present hypothesis in view of our observations on shrinkage of the body, and if we insert these figures into equation (3) of the Appendix, we find a shrinkage to 95 % within $7\frac{1}{2}$ sec. With formation of DHO the rate of shrinkage is practically the same.

The rate of vacuolar output returns ultimately to normal, within the limits of error, while the organism is still in $D_2O$. This is surprising, since if the diffusion constant for $D_2O$ is less than for $H_2O$ one would expect a corresponding difference in the rates of vacuolar output after equilibration unless some compensating effect had intervened. Actually in most of the experiments the reported rate of output was slightly higher in $D_2O$ (after equilibration) than in $H_2O$. This very fact emphasizes the need for caution. Although a standard error of only 0·08 $\mu^3$/sec. is applicable to the figures for Exp. 260359 (Table 1), a small systematic error in measurement would have a very considerable effect. Moreover, besides the unknown physico-chemical effects of the solutes within the organism on the activity of $D_2O$, there are unpredictable biological possibilities such as a metabolic effect of $D_2O$ on the internal solute concentration or an effect on the structure of the membrane itself.

Although we cannot exclude a purely kinetic interpretation of our results, there remains the possibility of an interpretation in terms of the equilibrium properties of $D_2O$. The lowering of activity of water within the organism by solutes is relatively small (concentration of pure water = 55·5 M, solute concentration = 0·05 osmolar). Thus a small difference in the fugacity might have a relatively large 'osmotic' effect. After equilibration, the osmotic differences due to solutes might be practically the same as in $H_2O$, so that a normal rate of output would be restored.

## SUMMARY

1. The suctorian *Discophrya collini* (Root) has been subjected to $D_2O$–$H_2O$ mixtures containing up to 99·7 % $D_2O$.

2. In 25 % $D_2O$ or over there is a rapid but temporary shrinkage of the body. This shrinkage is difficult to estimate owing to the wrinkling of the body surface, but amounts to at least 10 % in the undiluted (99.7 %) $D_2O$.

3. During the period of temporary shrinkage the contractile vacuole ceases activity. Normal activity is resumed when the normal volume is regained. In concentrations of $D_2O$ too low to cause shrinkage there is a temporary fall in the rate of vacuolar output.

4. Return to $H_2O$ leads to a brief but often very considerable rise in vacuolar output.

5. It is concluded that $D_2O$ penetrates less rapidly than $H_2O$. A difference of at least 10 % in the diffusion constants in the membrane would be required to explain our results. We cannot exclude this as unreasonable from our data, although an

explanation based on differences in the equilibrium properties of $D_2O$ and $H_2O$ might also be invoked.

We have received helpful comments from Dr J. Dainty (University of Edinburgh) and Prof. J. F. Danielli, F.R.S. (King's College, University of London). We are particularly glad to acknowledge the stimulating advice and valuable help of Dr A. Couper (Department of Chemistry, University of Bristol). We are also very much indebted to Dr M. H. Rogers (Department of Mathematics, University of Bristol) for the mathematical treatment of our problems in terms of diffusion, as given in the Appendix.

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## APPENDIX

BY M. H. ROGERS

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If it is assumed that no DHO is formed, so that the organism contains only $D_2O$ and $H_2O$ at any time, Fick's law of diffusion may be written as the pair of differential equations

$$\frac{dx}{dt} = -\frac{\alpha x}{V}, \tag{1}$$

$$\frac{dy}{dt} = \beta\left(1 - \frac{y}{V}\right), \tag{2}$$

where

$x$ = volume (in $\mu^3$) of $H_2O$ present at any instant;

$y$ = volume (in $\mu^3$) of $D_2O$ present at any instant;

$V$ = total volume at any instant;

$\alpha = D_{H_2O} A$;

$\beta = D_{D_2O} A$;

$A$ = surface area ($\mu^2$) of the cell;

and $D_{H_2O}$, $D_{D_2O}$ are Fick's diffusion constants for water and heavy water respectively. The equations have the solution

$$\frac{\alpha t}{V_0} = 1 - \frac{V}{V_0} - \lambda \log_e \left\{ \frac{V/V_0 - \lambda}{1 - \lambda} \right\}, \tag{3}$$

where $V_0$ is the original volume, $t$ the time in seconds measured from the instant of immersion in $D_2O$ and $\lambda$ is the ratio $D_{D_2O}/D_{H_2O}$. It follows immediately from this result that the final volume $V_\infty$ is simply $\lambda V_0$. Equation (3) gives the volume at any time; for instance, using the values for $\lambda$, $V_0$ and $\alpha$ given in the discussion above it is found that the volume decreases by 5 % in the first $7\frac{1}{2}$ sec.

When dissociation takes place the working is less simple, but it is possible to obtain a solution under the following assumptions:

I. All the incoming $D_2O$ reacts immediately with the $H_2O$ present to form DHO, so that there is no free $D_2O$ present in the organism until all the $H_2O$ has been used up. (This is an extreme case; in practice there will always be some $D_2O$ present in the organism.)

II. The diffusion coefficient for DHO is the arithmetic mean of those for $D_2O$ and $H_2O$.

The fundamental differential equations are now

$$\frac{dx}{dt} = -\frac{\alpha x}{V} - \beta, \tag{4}$$

$$\frac{dz}{dt} = -\frac{\alpha + \beta}{2} \frac{z}{V} + 2\beta, \tag{5}$$

where $z$ is the volume of DHO present at any instant. These equations are applicable up to the time $t_1$ when all the $H_2O$ in the organism has been used up; after this the process consists of the evacuation of DHO and the intake of $D_2O$, and is analogous to the process when no dissociation takes place. The solution in this case is conveniently written in the form

$$\frac{\tau}{\tau_0} \left\{ \frac{w-p}{w_0-p} \right\}^m \left\{ \frac{w-q}{w_0-q} \right\}^n = 1, \tag{6}$$

where the suffix zero denotes conditions at time $t = 0$ and the other symbols have the following definitions:

$$m, n = \tfrac{1}{2} \pm \frac{a}{2(p-q)}, \qquad a = \frac{\alpha(\lambda-3)}{2},$$

$$\tau = t + \frac{b}{c}, \qquad b = \frac{\alpha V_0 (1+\lambda)}{2},$$

$$w = \frac{V}{\tau}, \qquad c = \frac{-\alpha^2 (1-\lambda)^2}{2},$$

and $$p, q = \tfrac{1}{4}\alpha\{\lambda - 3 \pm \sqrt{(1 + 10\lambda - 7\lambda^2)}\}.$$

It is further found from equations (4) and (5) that

$$\frac{bx}{V_0} + \alpha z = c\tau, \tag{7}$$

and thus at time $t_1$, when $x = 0$

$$\alpha V_1 = c\tau_1. \tag{8}$$

Substitution of this result into equation (6) leads to a value of $V_1$, the volume at time $t_1$. The final volume $V_\infty$ is then given by the relation

$$V_\infty = \frac{D_{D_2O}}{D_{DHO}} V_1. \tag{9}$$

A few values of $V_\infty$ have been calculated, and it is seen that the difference between these and the corresponding values obtained from the theory ignoring dissociation is negligible and it is therefore unlikely that calculations based on a finite dissociation constant would yield significantly different results.

Table 2. *The final volume, expressed as a percentage of the original volume, calculated under the asssumptions I and II*

| $\frac{D_{D_2O}}{D_{H_2O}}$ | $\frac{V_\infty}{V_0}$ (%) |
|---|---|
| 0·9 | 89·7 |
| 0·85 | 84·2 |
| 0·8 | 78·6 |

# SODIUM REGULATION IN THE CRAYFISH *ASTACUS FLUVIATILIS*

## I. THE NORMAL ANIMAL

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### INTRODUCTION

It has long been established from osmotic pressure determinations that the freshwater crayfish *Astacus fluviatilis* controls the concentration of solutes in the blood quite rigidly at a level ($\Delta = -0{\cdot}80$ to $-0{\cdot}81°$ C.) far above that of the outside medium (e.g. Scholles, 1933; Schwabe, 1933). The concentrations of inorganic ions on which the blood osmotic pressure mainly depends have been measured on a number of occasions (e.g. Drilhon-Courtois, 1934; Bogucki, 1934). Of the two principal inorganic ions in the blood attention has been focused on the chloride rather than the sodium ion, which was less easily estimated until the advent of the flame photometer. It was found that the high blood chloride concentration remained constant even after weeks of starvation (Huf, 1933; Scholles, 1933) which indicated that food was not a primary source of chloride. Huf also showed that chloride was lost in distilled water and that the animals would eventually die if not replaced in fresh water. Krogh (1939) actually demonstrated that this recovery after distilled-water treatment involved the reabsorption of chloride over the body surface, presumably by the gills.

Schlieper & Herrmann (1930) first showed that the excretory organs produce urine which is markedly hypotonic to the blood, thus establishing their importance in maintaining the high blood osmotic pressure. This work was confirmed by that of Scholles (1933) and Picken (1936). Peters (1935) found that the chloride concentration of primary urine found in the coelomic sac and labyrinth of the excretory organ was the same as that of the blood, and fell as the urine passed down the nephridial canal to the bladder. This dilution of the urine was presumed to be by reabsorption of chloride rather than by secretion of water.

It appears that the blood chloride concentration is maintained under normal conditions by uptake over the body surface balancing losses over the body surface and in the urine. It is quite likely that the blood sodium concentration is maintained in a similar way. Recent work by Shaw (1959) on the effect of dilute sodium-chloride solutions and blood sodium concentration on the rate of uptake of sodium in *Astacus* has done much to reveal the properties of the mechanism in the body surface which is responsible. The work presented here and in future papers covers

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a similar field and is concerned with how the blood sodium concentration is kept constant by adjustments in the rates of uptake and loss of sodium effected by the body surface (gills) and excretory organs. As a first step, a study was made of sodium balance in the normal animal and these rates determined. The term 'normal animal' refers throughout to animals under steady-state conditions in artificial tap water containing 2 mM./l. NaCl. In many experiments $^{22}Na$ has been used to find the rates of loss and uptake of sodium. The validity of this method has been discussed by Shaw (1959) who gives evidence that under conditions where the transporting system approaches saturation about 20% of the isotope taken up may be due to 'exchange diffusion'. Evidence will be presented in subsequent papers (Bryan, 1960*a*, *b*) which indicates that although exchange diffusion of the particular form described by Ussing (1947) does not appear to exist, the mechanism responsible for sodium uptake acts to some extent as an exchange diffusion carrier which under normal conditions is responsible for about 30% of $^{22}Na$ movements.

## MATERIALS AND METHODS

### *Materials*

Male crayfish weighing up to 35 g. were used in nearly all experiments. The animals were obtained from the Freshwater Biological Association, Ambleside; L. Haig and Co. Ltd., Beam Brook, Newdigate, Dorking, Surrey and the Surrey Trout Farm, Nailsworth, Gloucestershire. They were kept in shallow running Bristol tap water in aquaria, sometimes for several months, and fed on blowfly larvae and earthworms. During the winter it was necessary to heat the aquaria to about 20° C., because at 10° C. the mortality rate was much higher. Animals were starved during experiments and for 2 or 3 days preceding them in order to allow any excess sodium obtained from the food to be removed.

$^{22}Na$ was obtained from the Radiochemical Centre, Amersham as sodium chloride solution.

### *Apparatus*

For experiments which involved taking blood samples from an animal at intervals over long periods of time, the tank shown in Fig. 1 A was used. It was made from sheet 'Perspex' and had a volume of 8·4 l. Two-thirds of the tank were covered by a removable lid which was fitted with two clamps for holding crayfish. When the tank was filled there was no air space beneath this lid. Detail of the crayfish clamp is shown in Figs. 1 B, C. The animal was clamped with the horizontal bar between the chelipeds and first pair of walking legs. The dorsal surface of the carapace fitted into the shaped hole in the lid so that the pericardial region was above the water level. Other tanks of similar design having volumes of 1, 2 and 9·6 l. were also constructed.

### *Blood sampling*

Blood samples of 2·6 $\mu$l. were taken for the measurement of radioactivity and sodium concentration. An 'Agla' micrometer syringe was used to which a silicone-lined nozzle made from 2 mm. bore 'Pyrex' tubing was attached by a piece of

rubber tube. The exposed carapace of the clamped animal was pierced in the pericardial region with a fine nickel-plated sewing needle. By carefully partially removing the needle, about 4 $\mu$l. of blood were allowed to escape on to the carapace. The 'Agla' syringe, filled with distilled water and clamped to an adjustable stand, was manoeuvred into position and the sample taken up.

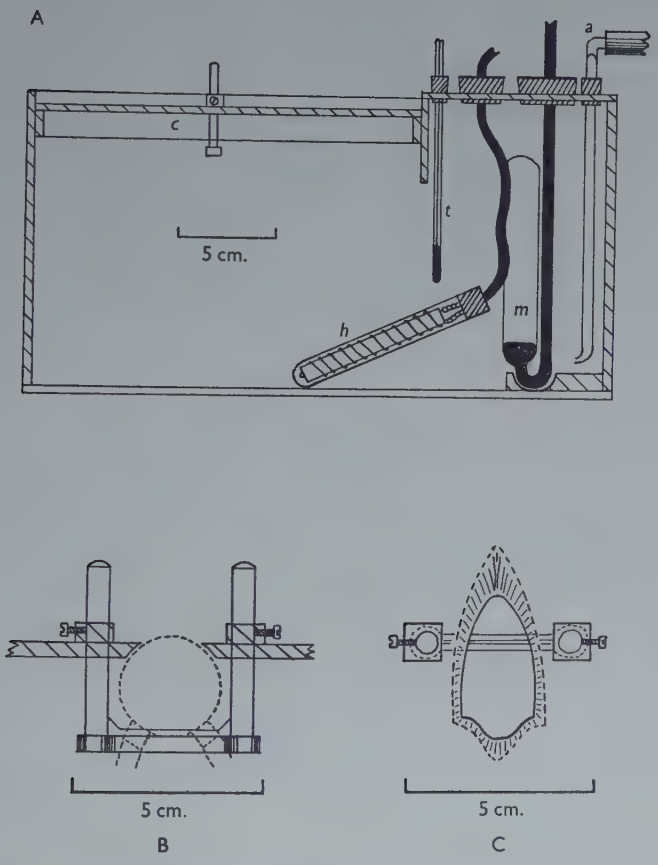


Fig. 1. 'Perspex' tank used in isotope experiments. (A) Side view: (*a*) air stirrer; (*c*) clamp; (*h*) heater; (*m*) thermoregulator; (*t*) thermometer. (B) Front view of clamp. (C) Top view of clamp.

For radioactive measurements the blood was washed from the syringe on to a planchet with 0·2 ml. of distilled water and the water was evaporated off at 60° C. The sample was counted with standard equipment incorporating a G.E.C. GM4 tube.

For the measurement of blood sodium concentration the sample was washed from the syringe into a small polythene tube containing 5 ml. of distilled water to give a final volume of 5·5 ml. The sodium concentration was determined by flame photometry. Blood samples of about 0·1 ml. were used for potassium determinations.

*Blood volume*

Blood volume was measured by injecting a known amount of the dye Evans blue (T-1824) into the circulation and observing its dilution. A modification of the method of Prosser & Weinstein (1950) was used. The animal was dried in a stream of air for 5 min., weighed and its body volume was measured by displacement of water in a measuring cylinder. Between 0·1 and 0·5 ml. of a 0·2% solution of the dye in artificial blood was injected into the pericardium of the clamped animal from an 'Agla' syringe at a rate of about 0·1 ml./min. Blood samples of about 0·25 ml. were taken from the base of a rear walking leg at 15, 30 and 50 min. after the injection and each delivered into 5 ml. of artificial blood. The optical density of these samples was compared with that of similar dye samples of known dilution to which blood from an uninjected animal had been added. Optical density was measured with a Hilger Spekker photo-electric absorptiometer. From a plot of optical density against time for each animal the density at zero time was obtained, and the corresponding blood volume calculated knowing the dilution of the standards and the volume of dye injected.

*Urine sampling*

Urine was collected in a braking pipette made from 2·5 mm. bore 'Pyrex' tube pulled out at the tip to less than 0·5 mm. diameter and operated by mouth suction. With the animal held ventral side upwards in the left hand, urine production was stimulated by touching the operculum covering the opening of the excretory organ with the tip of the pipette. If this was not sufficient to produce a flow of urine, slight lateral pressure was applied. When about 0·01 ml. had been collected it was blown out on to a polythene slide and a measured amount taken up with an 'Agla' syringe. It was diluted for sodium estimation in the same way as the blood sample.

*Urine volume*

As much urine as possible was removed from the animal, the anterior openings of the gill chambers were blocked with filter-paper and the animal was dried in a current of air. De Trey's dental cement was applied to the two excretory pores so as to form a solid block covering both papillae. The gill-chamber openings were unblocked and the animal was replaced in the solution for an hour before it was weighed. The drying procedure before weighing involved removal of excess water for 1 min. with a dry cloth and drying for 3 min. in a stream of air with the animal held vertically head downwards in a wide glass tube. At intervals the animal was reweighed. A number of curves showing the increase in weight of the animal with time are shown in Fig. 2. The increase in weight was relatively greater over the first few hours. This would be expected, as the increasing turgor pressure of the animal would tend to prevent the entry of more water. The volume of urine normally produced was therefore taken as the increase in weight over the first 6 to 8 hr. expressed as percentage increase per 24 hr.

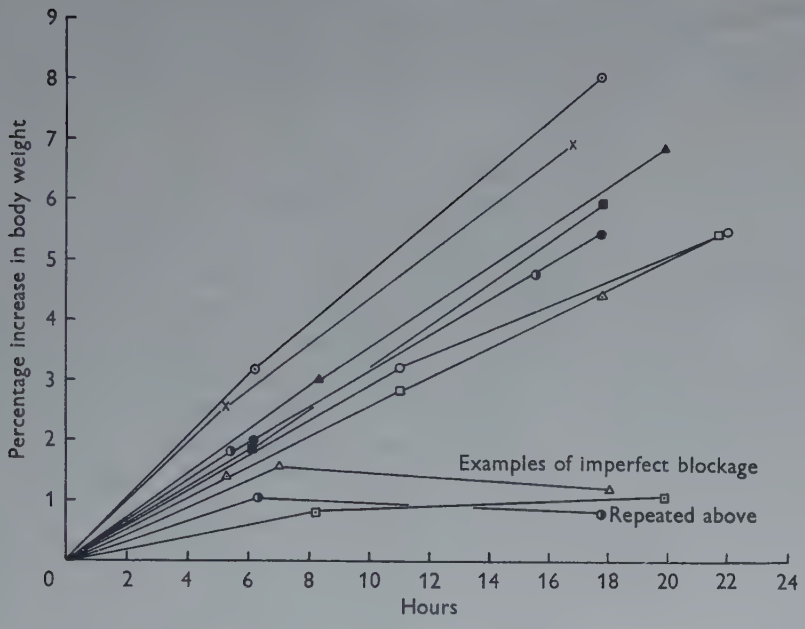


Fig. 2. Increase in weight of crayfish with blocked excretory pores.

*Potential difference across the surface of the animal*

Electrodes drawn out by hand from 1·5 mm. bore thick-walled glass tubing to a fine strong point and filled with 3 M-KCl-agar were used. They were connected to calomel electrodes by leads of 1 mm. bore Polythene tubing also filled with 3 M-KCl-agar. The animal was clamped in an insulated 'Perspex' tank of 2 l. capacity and the exposed dorsal surface was washed with distilled water and dried with compressed air. In some experiments the surface was smeared with silicone grease. One of the electrodes was introduced into the pericardium through a fine needle hole and the other dipped into the medium. The potential difference between the blood and outside medium was measured with a Cambridge pH and millivoltmeter and in later experiments with a 'Vibron' electrometer. After a steady potential difference had been reached it was noted and the electrode then removed and checked against that in the medium.

*Experimental solution*

A solution was used which had a composition similar to that of Bristol tap water except for the sodium concentration which was about five times as high:

| Salt | Concentration (mM./l.) |
|---|---|
| NaCl | 2·00 |
| KCl | 0·10 |
| $MgSO_4$ | 0·24 |
| $MgCO_3$ | 0·15 |
| $CaCO_3$ | 1·82 |

The carbonates were brought into solution as bicarbonate by saturating the distilled water with $CO_2$. The pH of this solution when aerated was about 7·8.

Except where otherwise stated, all experiments were performed at 20° C.

## THE BLOOD

### *Ionic composition of the blood*

The blood of *Astacus fluviatilis* has been analysed previously by several workers. As the estimation of sodium by chemical means was rather tedious only a few determinations were made. The discrepancy between these is seen in Table 1. During the present work a few measurements of blood potassium, in addition to sodium, were also made. The results which are for male animals kept in artificial tap water, usually at 20° C., are shown in Table 2. They agree fairly closely with the analyses by Drilhon-Courtois (1934) for male animals during the summer months when the crayfish would be expected to be active and well fed (Table 1).

Table 1. *Summary of blood analyses by previous workers*

| Author | Concentration of ions (mM./l.) Na | K | Ca | Mg | Cl | |
|---|---|---|---|---|---|---|
| Berger (1931) | — | 4·1 | 10·7 | — | 218 | — |
| Scholles (1933) | — | 5·2 | 10·4 | 2·6 | 195 | — |
| Bogucki (1934) | 152 | 3·1 | 12·0 | 2·5 | 175 | — |
| Drilhon-Courtois (1934) | 212 | 4·1 | 15·8 | — | — | ♂ May–June |
| | 220 | 5·0 | 14·0 | — | — | ♀ May–June |
| | 173 | 4·3 | 17·1 | — | — | ♂ Jan.–Feb. |
| | 175 | 5·1 | 15·6 | — | — | ♀ Jan.–Feb. |
| Huf (1934) | 185 | 7·8 | 7·7 | 1·5 | 199 | — |
| Peters (1935) | — | — | — | — | 196 | — |

Table 2. *Blood sodium and potassium concentrations*

| Ion | No. of determinations | Mean concentration (mM./l.) | Standard deviation |
|---|---|---|---|
| Na | 52 | 203 | 7 |
| K | 5 | 4·6 | 0·4 |

### *Blood volume*

The blood volumes of ten animals in three size groups were measured and are shown in Table 3 expressed as a percentage of the total volume. There is no evidence of the blood volume varying markedly with the size of the animal. The mean value of 29·2% agrees with the figure of 31% given by Prosser & Weinstein for *Cambarus virilis*.

It was sometimes necessary to convert the blood volume from percentage total body volume to percentage of body weight. For this purpose it was found that the mean specific gravity of forty-one male crayfish was 1·118 with a standard deviation of ±0·013.

Table 3. *Blood volume measured with Evans blue*

| Total body volume (ml.) | Blood volume (% body volume) |
|---|---|
| 36·5 | 31·2 |
| 33·0 | 25·9 |
| 32·5 | 27·5 |
| 25·5 | 32·4 |
| 24·2 | 24·2 |
| 24·0 | 31·6 |
| 20·5 | 31·7 |
| 17·4 | 29·3 |
| 14·0 | 23·6 |
| 14·0 | 33·6 |
| Mean | 29·2 |
| S.D. | 3·6 |

## THE URINE

### *Urine sodium concentration*

Some preliminary observations were made on animals in Bristol tap water (containing 0·4 mM./l. sodium) in aquaria. It was found that a group of animals actively feeding had a higher urine sodium concentration than a group not actively feeding yet not starved. At 18° C. the mean value for the group of eight feeding animals was 11·9 mM./l., the figures varying between 5·0 and 21·9 mM./l. At 16° C. the mean value for the group of six animals not actively feeding was 4·4 mM./l., the figures varying between 3·5 and 5·0 mM./l. The higher urine sodium concentrations found in feeding animals are probably due to the removal of excess sodium obtained from the food. The effect of starvation on urine sodium concentration was studied also. After heavy feeding, a group of animals was starved for up to 54 days. The urine sodium concentration of each animal was measured at intervals either until it died or until the end of the experiment, when its blood sodium concentration was measured. Experimental temperature varied between 12 and 15° C. The results are shown in Table 4. Some of the animals had a high urine sodium

Table 4. *The effect of starvation on the urine sodium and blood sodium concentrations of heavily fed animals in Bristol tap water at* 12–15° C.

| Starvation period (days) | Urine sodium concentrations and blood sodium concentrations (in brackets) of nine crayfish | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| 0 | 6·3 | 5·8 | 6·5 (201) | 18·3 | 11·7 | 5·7 | 11·2 (210) | 7·5 | 15·1 |
| 2 | 5·2 | 3·6 | — | 19·4 | 9·1 | 7·6 | — | 7·7 | 9·2 |
| 6 | 3·4 | 3·7 | — | 5·3 | 12·1 | 4·5 | — | 5·8 | 8·3 |
| 11 | 3·8 | 3·2 | — | 4·7 | 9·0 | 6·5 | — | 6·1 | 6·6 |
| 20 | 4·9 | 2·9 | — | 5·9 | 5·3 | 5·2 | — | 4·8 | 5·1 |
| 39 | Died at 30 days | 3·1 (202) | — | Died at 25 days | — | — | — | — | 5·8 (197) |
| 50 | — | — | — | — | 7·2 | — | — | 8·7 | — |
| 54 | — | — | — | — | 4·8 (199·5) | — | — | 7·8 (181·5) | — |

concentration initially which was presumably the result of heavy feeding. With starvation the urine concentration of these animals fell to a greater extent, so that after 20 days the figures for different animals were similar. The estimations of blood sodium concentration during the experiment show that starvation for this period does not have a very marked effect, as one animal was maintaining a concentration of 199·5 mM./l. even after 54 days. These analyses also indicate that the blood sodium concentration maintained in Bristol tap water is practically the same as that in artificial tap water.

When animals were transferred from Bristol tap water at 12–16° C. into artificial tap water at 20° C., the urine sodium concentration usually increased at first up to perhaps 20 mM./l. and then decreased after a few days to a fairly constant level similar to the original concentration. This may have been an effect of change in temperature.

In view of the results of these preliminary observations, urine sodium estimations in animals in artificial tap water at 20° C. were carried out over a period of days in starved animals until a more or less constant value was reached. The results in Table 5 are mean values of figures obtained when the concentration appeared to have become fairly constant. The mean urine sodium concentration of 6·0 mM./l. excludes the last three animals in the table. In the case of the last three animals the urine concentration rose rapidly from about 5 mM./l. in Bristol tap water to the high levels observed, which in this case were maintained without tending to fall. It has been suggested already that this effect may be due to the higher temperature of the experimental solution. In general the urine sodium concentrations in Bristol tap water (Table 4) and in artificial tap water (Table 5) are approximately

Table 5. *Urine sodium concentration in artificial tap water at* 20° C.

| No. | Period in artificial tap water before sampling (days) | Sampling period (days) | No. of samples | Mean urine sodium concentration: Right excretory organ mM./l. | Right excretory organ S.D. | Left excretory organ mM./l. | Left excretory organ S.D. |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 5 | 5 | 5·5 | 1·5 | 6·4 | 3·0 |
| 2 | 1 | 4 | 4 | 4·3 | 0·8 | 4·4 | 1·4 |
| 3 | 1 | 5 | 5 | 6·4 | 4·6 | 9·5 | 5·0 |
| 4 | 2 | 3 | 3 | 3·0 | 0·9 | 3·4 | 1·0 |
| 5 | 1 | 4 | 4 | 3·4 | 1·1 | 3·4 | 1·0 |
| 6 | 8 | 1 | 1 | 3·5 | — | 4·3 | — |
| 7 | 8 | 1 | 1 | 3·2 | — | 4·9 | — |
| 8 | 7 | 4 | 3 | 5·5 | 1·3 | 7·4 | 1·9 |
| 9 | 7 | 4 | 3 | 6·8 | 1·7 | 7·9 | 1·1 |
| 10 | 6 | 4 | 3 | 3·9 | 0·2 | 4·2 | 0·1 |
| 11 | 6 | 4 | 3 | 6·2 | 1·6 | 6·7 | 0·2 |
| 12 | 6 | 6 | 5 | 10·3 | 5·9 | 9·7 | 3·7 |
| 13 | 6 | 6 | 5 | 11·4 | 5·1 | 10·3 | 5·0 |
| 14 | 3 | 6 | 4 | 23·3 | 3·9 | 21·7 | 4·0 |
| 15 | 3 | 6 | 4 | 30·0 | 3·3 | 48·6 | 7·6 |
| 16 | 3 | 6 | 4 | 46·3 | 17·5 | 54·0 | 15·2 |
| Mean values not including last three results | | | | 5·7 | 2·7 | 6·3 | 2·4 |

Mean urine sodium concentration not including last three results 6·0 mM./l.

the same. In any one animal the urine sodium concentrations produced by the two excretory organs were similar but not always identical. Fluctuations of concentration always occurred simultaneously in the two organs so that there appeared to be a fairly constant relationship between them.

*Volume of urine produced in artificial tap water at* 20° C.

Two figures are quoted in the literature for the volume of urine produced per day in *Astacus fluviatilis*, 4% of the body weight per day from Scholles (1933) and 3·8% from Herrmann (1931). Both used the method of blockage with dental cement followed by the measurement of weight increase. The mean figure of 8·22% given in Table 6 is more than double the quoted figures. As the technique involves the need for perfect blockage a high result is more likely to be correct. Examples of faulty blockage are shown in Fig. 2. When one of these animals was reblocked a result similar to the others was obtained. When Herrmann's results were examined it was found that the quoted figure of 3·8% was the mean for two experiments in which the first weight increase had been measured after 24 hr. If the volume of urine is calculated from her figures, for animals whose excretory pores had been blocked for shorter times, the results bear a closer resemblance to those of the present work (Table 7). Herrmann's experiments were performed at 13–14° C. and her results might be expected to be rather lower.

Table 6. *Volume of urine produced in artificial tap water at* 20° C.

| Weight of animal (g.) | Increase in weight/24 hr. (%) |
|---|---|
| 34·5 | 7·68 |
| 21·7 | 12·00 |
| 27·2 | 11·75 |
| 25·1 | 6·62 |
| 43·3 | 8·29 |
| 35·1 | 8·86 |
| 37·7 | 8·45 |
| 24·0 | 8·15 |
| 19·2 | 7·07 |
| 28·9 | 6·40 |
| 18·9 | 5·14 |
| Mean | 8·22 |
| S.D. | 2·10 |

Table 7. *Volume of urine produced in tap water at* 13–14° C. *calculated from Herrmann* (1931)

| Time of blockage (hr.) | 2 | 5 | 12 | 16 | 20 |
|---|---|---|---|---|---|
| No. of animals | 4 | 3 | 13 | 7 | 5 |
| Mean % increase in weight/24 hr. | 7·7 | 10·3 | 5·2 | 5·0 | 6·8 |

## THE EXCHANGE OF $^{22}Na$ IN NORMAL ANIMALS

### *Theoretical considerations*

The equations used to calculate transfer constants from data obtained in experiments using $^{22}Na$ were those of Harris & Burn (1949) for a single cell or a one-compartment system.

If a cell is in ionic equilibrium with the outside medium then the influx of sodium is equal to the outflux and

$$k_{in}[Na_{out}] = k_{out}[Na_{in}], \tag{i}$$

where $k_{in}$ and $k_{out}$ are the transfer constants into and out of the cell and $[Na_{out}]$ and $[Na_{in}]$ the external and internal sodium concentrations. When the outside medium is labelled with $^{22}Na$ the rate at which it enters and leaves the cell depends on the same transfer constants as does the penetration of inactive sodium. If all the cell sodium is exchangeable, then at infinite time when the numbers of $^{22}Na$ ions passing in either direction across the membrane are equal

$$k_{in}[Na^*_{out}] = k_{out}[Na^*_{in}]_{t=\infty}, \tag{ii}$$

where $[Na^*_{out}]$ is the concentration of $^{22}Na$ in the medium and $[Na^*_{in}]_{t=\infty}$ is the internal concentration of $^{22}Na$ at infinite time calculated from the sodium concentration of the cell using

$$\frac{[Na_{in}]}{[Na_{out}]} = \frac{[Na^*_{in}]_{t=\infty}}{[Na^*_{out}]}. \tag{iii}$$

In order to find $k_{out}$ two methods may be used: (*a*) The cell is placed in a solution containing sodium labelled with $^{22}Na$ and the uptake of isotope is measured by the concentration in the cell at intervals of time, $[Na^*_{in}]_t$, used in the equation

$$-k_{out}t = \ln\left(1 - \frac{[Na^*_{in}]_t}{[Na^*_{in}]_{t=\infty}}\right). \tag{iv}$$

$$\log_{10}\left(1 - \frac{[Na^*_{in}]_t}{[Na^*_{in}]_{t=\infty}}\right)$$

is plotted against time $t$ and the slope of the straight line multiplied by 2·303 gives the value of $k_{out}$. (*b*) A cell already containing $^{22}Na$ is placed in isotope-free medium and the loss of isotope from the inside observed with time. In this case $[Na^*_{in}]_t$ is used in the equation

$$-k_{out}t = \ln\left(\frac{[Na^*_{in}]_t}{[Na^*_{in}]_{t=0}}\right). \tag{v}$$

$$\log_{10}\left(\frac{[Na^*_{in}]_t}{[Na^*_{in}]_{t=0}}\right)$$

is plotted against time and the slope of the straight line multiplied by 2·303 gives the value of $k_{out}$. $k_{out}$ has the dimensions hr.$^{-1}$. The outflux or influx of sodium at equilibrium is found from equation (i):

$$\text{influx} = \text{outflux} = k_{out}[Na_{in}] \text{ mM./l. of cell content/hr.}$$

Sodium entering or leaving the blood of a whole crayfish, in contrast to a single cell, must pass through at least two cell membranes and the system may be further complicated by exchange between the blood and other tissues. Experiments were performed to see how closely the crayfish approximated to a one-compartment system and if possible to find values for $k_{out}$ using the general methods and equations outlined above for a single cell.

### *The exchangeability of sodium in the blood*

Two crayfish were left in 4 l. of artificial tap water containing 20 $\mu$c. of $^{22}$Na for 1080 hr. The activity and sodium concentration of the blood and outside medium were then measured. If all the sodium in the blood is exchangeable then at equilibrium equation (iii) will hold. The observed concentration ratios in Table 8 show that allowing for errors practically all the blood sodium has exchanged by 1080 hr.

Table 8. *Blood/medium ratios for inactive sodium and* $^{22}$Na *at equilibrium*

| Animal | $\frac{[Na_{in}]}{[Na_{out}]}$ | $\frac{[Na^*_{in}]_{t=1080\text{ hr.}}}{[Na^*_{out}]}$ |
|---|---|---|
| 1 | 63·1 | 68·7 |
| 2 | 115·0 | 112·0 |

### *The uptake of* $^{22}$Na *from artificial tap water*

Animals were clamped in the 8·4 l. tank containing artificial tap water to which 50 $\mu$c. of $^{22}$Na had been added. Blood samples were taken at intervals for radioactive and sodium concentration measurements. During the experiment small amounts of $^{22}$Na were added to the medium to compensate for that taken up by the animal. A typical curve for uptake of $^{22}$Na is shown in Fig. 3. The corresponding semi-logarithmic plot in Fig. 4 gives a straight line over the first 300 hr. from which $k_{out}$ was found. The later deviation from this is due to the fall in blood sodium concentration because $[Na^*_{in}]_{t=\infty}$ was calculated from the initial sodium concentration. It is probable that fouling of the medium was responsible for the fall in blood sodium concentration.

### *The loss of* $^{22}$Na *from the blood into artificial tap water*

Radioactive animals were obtained by placing them in 1 l. of Bristol tap water containing 10 $\mu$c. of $^{22}$Na. The high specific activity of this medium made it possible to obtain a blood activity of 500–1000 counts/min./2·6 $\mu$l. in 2 or 3 days. The loss of $^{22}$Na from the blood was determined in 8·4 l. of artificial tap water and the solution was changed every 2 days. A typical curve showing loss of blood activity with time is shown in Fig. 5. In this case no fouling of the medium was encountered and the blood sodium concentration did not fall. The semi-logarithmic plot from which $k_{out}$ was found is shown in Fig. 6. This particular experiment also shows that the moulting cycle does not appear to affect sodium balance until 48 hr. prior to the moult.

Values of $k_{out}$ obtained by both methods are given in Table 9. When the constancy of the blood sodium concentration is considered $k_{out}$ shows considerable variation. No relationship is apparent between $k_{out}$ and the original source of the animals, their size or the time of year, although figures for animals which were about to moult (signified by $M$) are rather higher than the mean.

It can be concluded that with regard to the transfer of $^{22}Na$ between the blood and artificial tap water the animal acts like a one-compartment system.

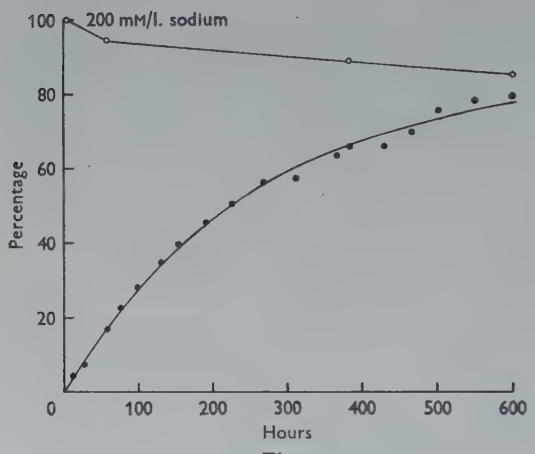


Fig. 3

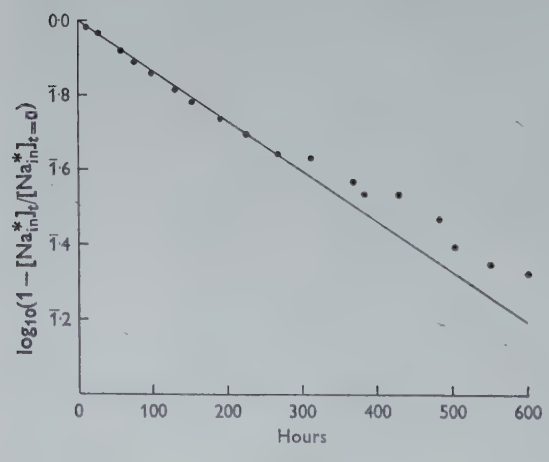


Fig. 4

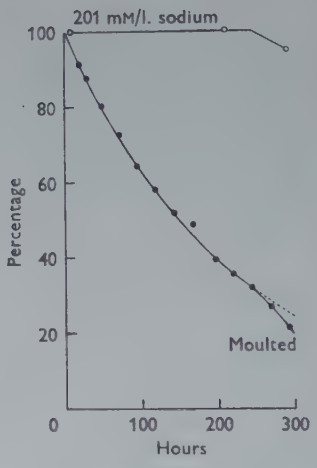


Fig. 5

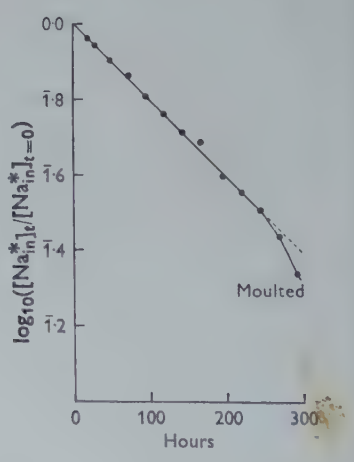


Fig. 6

Fig. 3. Uptake of $^{22}Na$ from artificial tap water by a normal animal. ●, blood radioactivity; ○, blood sodium concentration.

Fig. 4. Radioactive results from Fig. 3 plotted according to equation (iv).

Fig. 5. Loss of $^{22}Na$ from a normal animal into artificial tap water. ●, blood radioactivity; ○, blood sodium concentration.

Fig. 6. Radioactive results from Fig. 5 plotted according to equation (v).

Table 9. *Summary of values for* $k_{out}$ *in artificial tap water at* 20° C.

| Date | Method | Weight (g.) | Blood sodium concentration (mM./l.) | $k_{out}$ hr.$^{-1}$ |
|---|---|---|---|---|
| 24. i. 56 | Uptake | 36·0 | 205 | 0·00383 |
| 4. ii. 58 | Loss | 28·5 | 200 | 0·00560 |
| 14. ii. 58 | Loss | 21·5 | 193 | 0·00571 |
| 11. ii. 57 | Loss | 26·0 | 213 | 0·00551 (*M*) |
| 14. iv. 58 | Loss | 19·5 | 197 | 0·00254 |
| 30. iv. 56 | Uptake | 39·0 | 200 | 0·00502 |
| 3. v. 58 | Loss | 27·5 | 189 | 0·00427 (*M*) |
| 11. v. 58 | Loss | 20·0 | 203 | 0·00434 |
| 27. v. 56 | Uptake | 32·6 | 200 | 0·00327 |
| 27. v. 56 | Uptake | 31·3 | 204 | 0·00473 |
| 23. vii. 58 | Loss | — | 208 | 0·00760 (*M*) |
| 20. viii. 57 | Loss | — | 215 | 0·00328 |
| 25. viii. 56 | Loss | 32·0 | 202 | 0·00457 (*M*) |
| 1. x. 57 | Loss | 23·7 | 208 | 0·00228 |
| 1. x. 57 | Loss | 22·5 | 200 | 0·00397 |
| 11. xi. 56 | Uptake | 39·1 | 211 | 0·00359 |
| 12. xi. 56 | Uptake | 41·4 | 211 | 0·00215 |
| 24. xi. 56 | Uptake | 32·8 | 206 | 0·00398 |
| 24. xi. 56 | Uptake | 34·6 | 217 | 0·00550 |
| | | Mean value | 204 | 0·00428 |
| | | S.D. | 7 | 0·00135 |

### *The exchange of* $^{22}$Na *with the tissues*

If $^{22}$Na is exchanged between the blood and other tissues then the size of the compartment exchanging sodium with the medium will be considerably larger than the blood volume. It was measured by placing one or two animals in 1 l. of Bristol tap water containing 10 $\mu$c. of $^{22}$Na and comparing the depletion in the activity of the medium with the gain in the activity of the blood:

Volume of compartment having the sodium concentration of blood (sodium space)

$$V' = \frac{\text{vol. of medium} \times \text{fall in activity of medium}}{\text{gain in activity of blood}}.$$

Values of $V'$ are given in Table 10.

It can be calculated that the difference of 18% of the body volume between $V'$ and the blood volume as measured with Evans blue is equivalent to complete exchange having taken place between the blood (Na = 200 mM./l.) and tissues having a mean sodium concentration of 51 mM./l. The mean sodium concentration of the tissues is not known. That of the muscles is about 35 mM./kg. $H_2O$ and judging from the chloride analyses of Scholles (1933) that of the digestive gland would be about 100 mM./kg. $H_2O$. A figure of 51 mM./l. for tissue sodium concentration is therefore not inconsistent with complete exchange having taken place between blood and tissues. It has been found by Shaw (1958) that in *Carcinus maenas* exchange of $^{24}$Na between blood and muscle fibres has a half time of 2–3 min. A similar figure in *Astacus* would give almost complete equilibration of $^{22}$Na between blood and tissues at all blood activities. Thus together they act as

a single compartment having a volume of 46·9% of the body volume and the sodium concentration of the blood.

Table 10. *Sodium space as measured with* $^{22}Na$

| Exp. | No. of animals | Body volume (ml.) | Sodium space $V'$ as % body volume |
|---|---|---|---|
| 1 | 1 | 20·5 | 43·0 |
| 2 | 1 | 18·0 | 41·5 |
| 3 | 1 | 19·0 | 56·5 |
| 4 | 1 | 25·5 | 42·1 |
| 5 | 2 | 17·3 | 47·4 |
| — | — | 23·6 | 47·4 |
| 6 | 2 | 24·5 | 47·9 |
| — | — | 17·8 | 47·9 |
| 7 | 2 | 17·8 | 43·4 |
| — | — | 21·3 | 43·4 |
| 8 | 2 | 22·5 | 51·0 |
| — | — | 23·7 | 51·0 |
| | | Mean | 46·9 |
| | | S.D. | 4·3 |

*A comparison of sodium losses over the body surface with those in the urine*

Total sodium outflux $= k_{out}[Na_{in}]$.

Mean value of $k_{out}$ = 0·00428 hr.$^{-1}$.

Mean blood sodium concentration $[Na_{in}]$ = 204 mM./l.

Total sodium outflux as measured with $^{22}Na$ = 0·870 mM./l. blood/hr.

Mean urine sodium concentration = 6·0 mM./l.

Mean volume of urine produced = 8·22% body weight/24 hr.

Mean specific gravity of crayfish = 1·118.

Size of compartment with sodium concentration of blood $V'$ (sodium space) = 46·9% of body volume.

Amount of sodium lost in urine $= \dfrac{6{\cdot}0 \times 8{\cdot}22 \times 1{\cdot}118}{46{\cdot}9 \times 24}$.

= 0·049 mM./l. blood/hr.

Percentage outflux contributed by urine = 5·6.

*Potential difference across the surface of the animal*

The only certain criterion of active transport of ions is transport from a lower to a higher electrochemical potential (Rosenberg, 1954). In order to explain the maintenance of a blood sodium concentration of 200 mM./l. in Bristol tap water containing 0·4 mM./l. sodium without evoking active transport, a potential difference of 58 log 200/0·4 or 156 mV. (negative with respect to the blood) would be needed across the surface of the animal. A similar potential difference of the reverse sign would be required to maintain the blood chloride concentration passively.

The potential difference between the blood and the medium was measured in Bristol tap water and artificial tap water. Readings at intervals of several hours and sometimes days gave only small potential differences which were fairly constant. Ten animals in Bristol tap water gave a mean figure of 6·6 mV. (positive with respect to the blood) with a standard deviation of ±4·8 mV. Twelve animals in artificial tap water gave a mean value of 4·1 mV. with a standard deviation of ±3·2 mV. Although the possibility of a leakage of potential difference cannot be entirely ruled out, it is unlikely that this would take place across the exposed dry surface of the carapace which was sometimes smeared with silicone grease as a further precaution. The potential differences which were found fall so far short of those required to explain a passive distribution of sodium or chloride that active transport inwards of both these ions seems likely.

## DISCUSSION

Uptake and loss of $^{22}Na$ by the blood of *Astacus* under steady state conditions is fairly closely described by equations derived for a one-compartment system. In the crayfish there is probably a rapid exchange of sodium between the blood and tissues, with the result that although their sodium concentrations are different they act as a single compartment when exchange with the external medium is considered. The volume of this compartment having the sodium concentration of the blood (sodium space) exceeds the blood volume as measured with Evans blue by an amount roughly equal to the sodium in the tissues.

When the animal is in a steady state its blood sodium concentration of about 203 mM./l. is maintained by sodium uptake balancing losses through the body surface and in the urine. It has been assumed for the purposes of calculations by Krogh (1939) and Potts (1954) that extra-renal salt loss is negligible. The present work with $^{22}Na$ indicates that the reverse is nearer the truth, as renal losses constituted only 5·6% of total sodium outflux. It is possible that the value for total sodium outflux of 0·87 mM./l. blood/hr. measured with $^{22}Na$ may, due to a type of 'exchange diffusion', be about 30% higher than the actual tendency for the animal to lose sodium (Bryan, 1960*a*). Urine losses would represent about 8% of this lower figure. This supports the estimate of 10% made by Shaw (1959). Temporary increases in the urine sodium concentration of several times normal were sometimes found in heavily fed animals and those subjected to a sudden increase in temperature and external sodium concentration. This appears to indicate the importance of the excretory organs in restoring sodium balance under conditions where the blood sodium concentration might be expected to have increased slightly.

As a result of potential difference measurements across the body surface it has been suggested that both sodium and chloride are actively absorbed, probably by the gills. This is not inconsistent with observations made by Maluf (1940) and Schmidt-Nielsen (1941). In salt-depleted animals they demonstrated the net uptake of chloride without potassium from dilute KCl solution and the uptake of sodium without the corresponding anion from $Na_2SO_4$ solution. From dilute NaCl

solutions equal amounts of sodium and chloride were taken up. This work indicates the existence of separate sodium and chloride transport mechanisms which can act independently to some extent, but which work at about the same rate in a solution of both ions. If under normal conditions sodium and chloride were transported at equal rates then a large potential difference across the body surface would not be expected. In this connexion Jørgensen, Levi & Zerahn (1954) working with live anurans showed that under some conditions both sodium and chloride were actively transported and that the mechanisms were able to work independently.

## SUMMARY

1. In Bristol tap water containing 0·4 mM./l. sodium and artificial tap water containing 2 mM./l. sodium, *Astacus* maintains a blood sodium concentration of about 203 mM./l. This value was not markedly affected by starvation periods of up to a month.

2. Methods of taking small blood and urine samples from individual crayfish at intervals over several hundred hours have been described.

3. Under steady state conditions, curves for the uptake and loss of $^{22}Na$ by the blood are described by equations derived for a one-compartment system.

4. The volume of this single compartment, which exchanges sodium with the medium, is larger than the actual blood volume by an amount roughly equivalent to the sodium in the tissues. Exchange of sodium between the blood and tissues is probably very rapid.

5. Sodium losses in the urine account for about 6% of the total sodium outflux found using $^{22}Na$. The urine sodium concentration of about 6 mM./l. was temporarily increased by conditions such as heavy feeding when the blood may have gained additional sodium.

6. Potential difference measurements across the body surface indicate that the high blood sodium concentration is maintained by active uptake of sodium.

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# SODIUM REGULATION IN THE CRAYFISH *ASTACUS FLUVIATILIS*

## II. EXPERIMENTS WITH SODIUM-DEPLETED ANIMALS

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(*Received* 15 *August* 1959)

### INTRODUCTION

The net uptake of ions from dilute solutions by crayfish previously depleted by exposure to distilled water, first demonstrated by Krogh (1939), has been studied by Maluf (1940) and Schmidt-Nielsen (1941). Their results indicated that under these conditions sodium was reabsorbed at about the same rate as chloride from dilute solutions of sodium chloride. If one of these ions was absent from the outside solution the other was still taken up, showing that the uptake mechanisms are to some extent independent.

In this paper the loss of sodium in distilled water and the subsequent net uptake of sodium from artificial tap water has been studied with the aid of $^{22}Na$. Information has been obtained as to the way in which the gills and excretory organs of *Astacus* combine to correct fluctuations below the normal blood sodium level.

Maluf (1940) and Schmidt-Nielsen (1941) also described experiments which it was claimed showed that the staining of the gills by silver from dilute silver nitrate solution was an active process requiring energy. Their work also showed that staining was limited to the more basal parts of the gills. These experiments followed the demonstration by Koch (1934, 1938) that in the larvae of *Culex* and *Chironomus* the organs which are stained by silver also absorb salt. Croghan (1958*a*) showed that in *Artemia* silver staining of the branchiae was a passive process and suggested that this was also true for other animals. Silver staining in the gills of *Astacus* has been re-examined in conjunction with other experiments which identify the gills as the main site of sodium uptake.

Most of the experimental methods used in this work have already been described (Bryan, 1960*a*). Unless otherwise stated the artificial tap water used contained 2 mM./l. NaCl and the experimental temperature was 20° C.

### THE EFFECT OF DISTILLED WATER ON SODIUM BALANCE

#### *The effect on the blood*

Experiments were performed in which blood samples were taken at approximately 12 hr. intervals in animals (one or two together) clamped in a 2 l. capacity tank through which glass-distilled water was perfused at a rate of 500 ml./hr. from a

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Krogh constant flow apparatus (Krogh, 1939). The type of curve described by the fall in blood sodium concentration is shown in Fig. 1.

### *The effect on the urine*

An experiment corresponding to those above showed that in distilled water the urine sodium concentration fell rapidly from about 8 to 1 mM./l. when it remained fairly constant (Fig. 2). As the volume of urine produced was about the same as normal (i.e. 8·22% body weight/24 hr.) the rate of loss of sodium via the excretory organs had been reduced to about one-eighth the normal value of 5·6% of total sodium outflux as shown by $^{22}$Na (Bryan, 1960*a*).

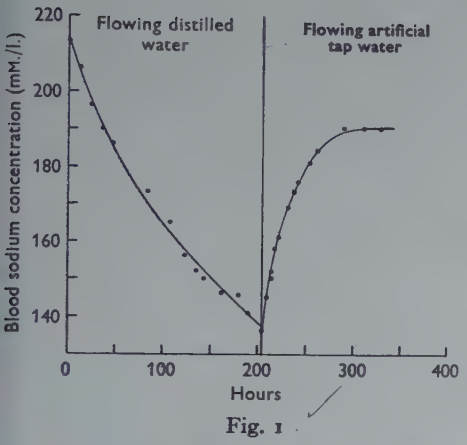


Fig. 1

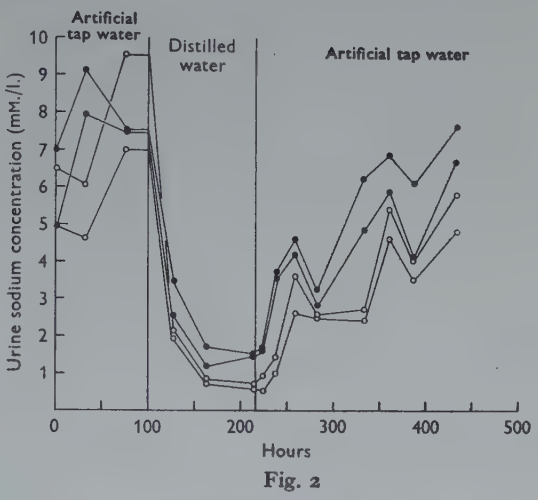


Fig. 2

Fig. 1. Loss of sodium from the blood in flowing glass-distilled water and subsequent net uptake from flowing artificial tap water.

Fig. 2. The effect of flowing glass-distilled water and subsequent return to artificial tap water on the sodium concentration of urine produced by both excretory organs of two animals.

### *The effect on the rate of loss of* $^{22}$Na

Single animals clamped in the 8·4 l. capacity tank were used in these experiments. After a control period when the rate of loss of $^{22}$Na from the blood into artificial tap water was determined in the way described previously (Bryan, 1960*a*), glass-distilled water was used as the outside medium and changed twice a day. The blood sodium concentration was measured at intervals throughout the experiment in order to correlate the level of $^{22}$Na with the total sodium concentration. Two results for the loss of $^{22}$Na are given in Fig. 3 as a plot of $\log_{10}([Na^*_{in}]_t/[Na^*_{in}]_{t=0})$ against time and represent extreme cases. To show that the net loss of sodium from the blood corresponds with the loss of $^{22}$Na, figures for blood sodium concentration in the distilled water were plotted in the same way. The actual blood sodium concentrations at the beginning and end of the period in distilled water are also shown. The values for $k_{out}$ in artificial tap water and distilled water for these Exps. (1, 2) and other similar Exps. (3, 4) are given in Table 1.

As the rate of loss of $^{22}Na$ in distilled water corresponded with the net loss of sodium from the blood some values of $k_{out}$ were found from the latter observations only (Exps. 5–9, Table 1). In experiments such as those on the loss and uptake of $^{22}Na$ during net uptake of sodium by depleted animals, the time of depletion in

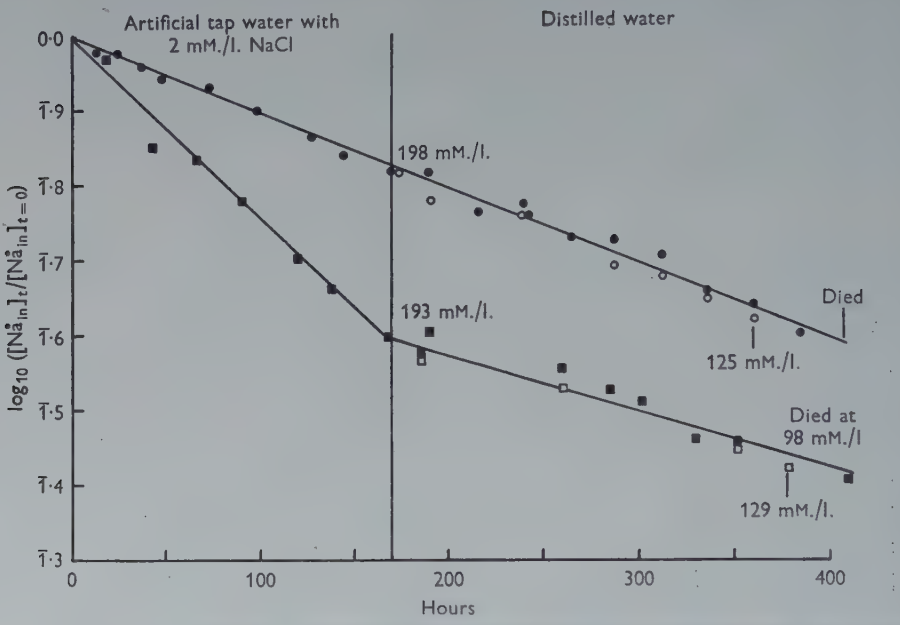


Fig. 3. Two experiments showing the effect of glass-distilled water on the loss of $^{22}Na$ and on the blood sodium concentration. ●, ■, fall in blood radioactivity plotted according to equation (v); ○, □, fall in blood sodium concentration plotted in the same way. Actual blood sodium concentrations before and after exposure to distilled water are also shown.

Table 1. *The effect of distilled water on* $^{22}Na$ *outflux*

| Exp. | $k_{out}$ hr.$^{-1}$ artificial tap water | $k_{out}$ hr.$^{-1}$ distilled water | $k_{out}$ D.W./$k_{out}$ T.W. × 100 |
|---|---|---|---|
| 1 | 0·00257 | 0·00257 | 100·0 |
| 2 | 0·00556 | 0·00177 | 31·8 |
| 3 | 0·00208 | 0·00166 | 80·0 |
| 4 | 0·00366 | 0·00170 | 46·5 |
| 5 | — | 0·00224 | — |
| 6 | — | 0·00230 | — |
| 7 | — | 0·00253 | — |
| 8 | — | 0·00150 | — |
| 9 | — | 0·00167 | — |
| 10 | 0·00435 | 0·00401 | 92·2 |
| 11 | 0·00421 | 0·00188 | 44·6 |
| 12 | 0·00514 | 0·00396 | 77·2 |
| 13 | 0·00274 | 0·00144 | 52·6 |
| 14 | 0·00442 | 0·00165 | 37·4 |
| 15 | 0·00346 | 0·00285 | 82·5 |
| Mean (excluding 5–9) | 0·00382 | 0·00235 | 64·5 |

distilled water and the blood sodium concentration finally reached were both known. Assuming that the initial sodium concentration was 203 mM./l., $k_{out}$ in distilled water could be calculated and compared with the figure obtained using $^{22}Na$ later in the experiment when the animal was again in a steady state in artificial tap water (Exps. 10–15, Table 1).

Table 1 shows that in general sodium was lost more slowly from the animal into distilled water than was $^{22}Na$ into artificial tap water. A reduction of about 5% would be expected as a result of the fall in losses via the excretory organs. However, the mean reduction of $k_{out}$ was about 36%, of which presumably 31% was due to the body surface.

## THE EFFECT OF REDUCING THE SODIUM CONCENTRATION OF ARTIFICIAL TAP WATER

### *Artificial tap water with* 0·5 mM./l. NaCl

$k_{out}$ as measured by the rate of loss of $^{22}Na$ was found to be approximately the same in this solution as in normal artificial tap water with 2 mM./l. NaCl. The blood sodium concentration remained the same in both solutions and therefore presumably the rate of uptake of sodium remained constant.

### *Artificial tap water with* 0·02 mM./l. NaCl

This experiment was performed in the same way as those described for the measurement of $k_{out}$ in distilled water. The artificial tap water containing 0·02 mM./l. NaCl was changed each day. The results shown in Fig. 4 are plotted in the same way as those in Fig. 3 so that the loss of $^{22}Na$ into the low sodium medium is directly compared with the net fall in blood sodium concentration. After an initial fall the blood sodium concentration started to level out at about 168 mM./l. when presumably the animal was able to take up sufficient sodium from the low external concentration to balance the lower rate of loss.

When animals were kept in artificial tap water which contained little or no sodium and was changed daily, it was found that after 4 days the urine sodium concentration had fallen from about 5 to 1 mM./l. As was the case in distilled water, reduced loss of sodium via the excretory organs could not account for the total fall in outflux as shown by $^{22}Na$.

## THE REABSORPTION OF SODIUM BY DEPLETED ANIMALS

### *The net uptake of sodium*

Animals which had been 'washed out' in distilled water for 7–8 days were clamped in a tank of artificial tap water and blood samples taken at intervals for sodium estimation. A typical result is shown in Fig. 1. The animal took up sodium rapidly but did not regain all the sodium which had been lost. During the uptake process the weight of the animal did not change by as much as 1% so that concentration changes could not be attributed to any change of volume. The actual curve of net uptake was found to be roughly exponential in character, so that a plot of

$\log_{10}(1-(\text{Na gained})_t/(\text{Na gained})_{t=\infty})$ against time gave a fairly straight line (Fig. 5). $(\text{Na gained})_t$ is the net increase in blood sodium concentration after time $t$ in artificial tap water and $(\text{Na gained})_{t=\infty}$ the net increase in sodium concentration when a steady state had been reached. This indicates that the net uptake process is governed by a rate constant related to the slope of the straight line. It was possible to compare the rate of the net uptake process in different animals (Fig. 5). Some of the straight lines in Fig. 5 are taken from the results shown in Figs. 1, 6 and 7 and the appropriate symbols are used. The figures associated with the lines in Fig. 5 are the blood sodium concentrations prior to net uptake and the times in distilled water taken to achieve them. From these results and others not shown here it appears that the rate of net uptake is lower as the degree of sodium depletion is increased and is not related to the length of time in distilled water.

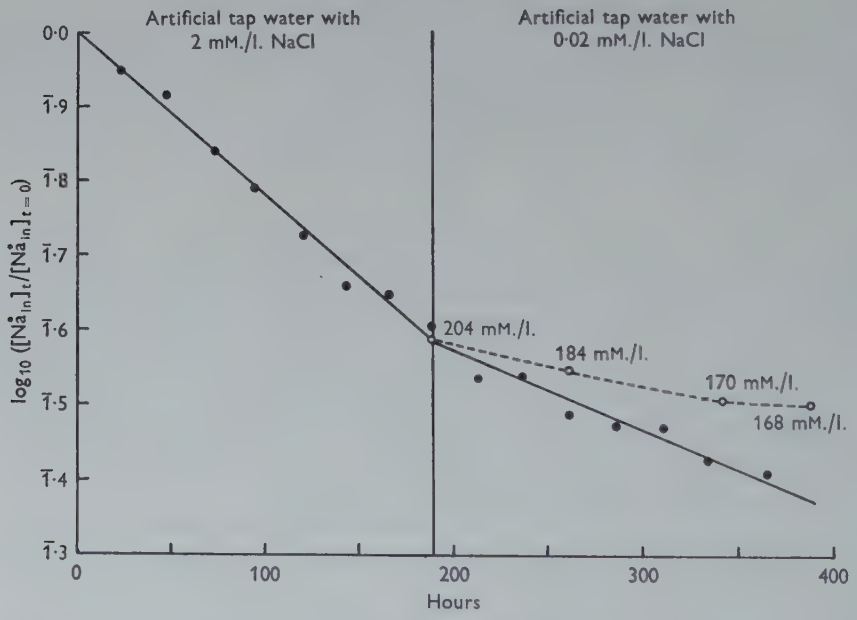


Fig. 4. Effect of artificial tap water containing 0·02 mM./l. NaCl on the loss of $^{22}Na$ and on the blood sodium concentration. ●, fall in blood radioactivity plotted according to equation (v); ○, fall in blood sodium concentration plotted in the same way. Actual blood sodium concentrations are also given.

While the blood sodium concentration was increasing in artificial tap water, the urine sodium concentration was increased to a figure slightly below the original level (Fig. 2).

In searching for some form of control of sodium balance the possibility of hormones was considered. As the eyestalk is an important endocrine region in *Astacus* the effect of eyestalk removal on the net uptake of sodium by depleted animals was studied. In some cases the eyestalks were ligatured with nylon thread and in others completely removed after first ligaturing. This treatment did not,

however, have any effect on the net uptake curve and equilibrium was reached in the usual way. Later, however, the animals always died and this was preceded by a fall in blood sodium concentration. In six experiments the times taken for animals to die were 10, 7, 12, 24, 8 and 11 days. It seems probable that the eyestalks do not exert any direct control over sodium balance. The fall in blood sodium concentration prior to death seems more likely to be an indirect result of the removal of some other necessary factor.

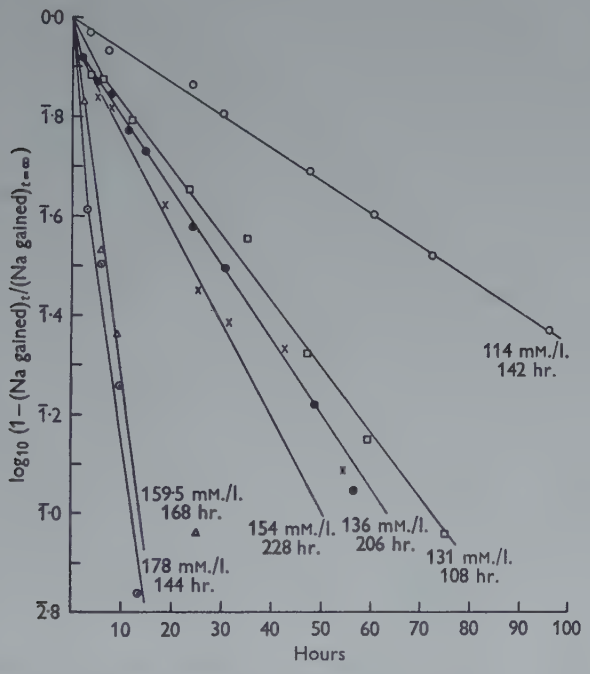


Fig. 5. A comparison of net uptake rates. The figures show the level to which blood sodium had been depleted and the time in distilled water taken to achieve this.

Measurements of potential difference across the body surface during the initial phase of net uptake from artificial tap water gave a mean figure of 9·2 mV. with a standard deviation of ±4·1 mV. for four animals. This value is only slightly greater than the figure of 4·1 mV. (positive with respect to the blood) found in normal animals which was taken to imply that both sodium and chloride were actively transported inwards at similar rates (Bryan, 1960*a*).

### *The uptake of* $^{22}$Na *during the net uptake of sodium*

These experiments were performed in the same way as those involving the uptake of $^{22}$Na in normal animals (Bryan, 1960*a*). In addition it was necessary to keep the sodium concentration of the medium constant during the net uptake. Samples of medium for sodium analysis were taken every few hours in the early stages of the experiment and any fall in concentration was corrected by adding

NaCl. A typical result showing the simultaneous changes in sodium concentration and blood activity is given in Fig. 6. As with the normal animal the $^{22}Na$ results are also plotted according to equation (iv) in Fig. 8 (i.e. as a plot of $\log_{10}(1-[Na^*_{in}]_t/[Na^*_{in}]_{t=\infty})$ against time). In the initial stages of $^{22}Na$ uptake, most of the isotope moves in the direction out to in and should correspond directly to the amount of sodium entering the animal. The broken line in Fig. 6 shows the path of $^{22}Na$ uptake in the normal animal and was constructed from the value of

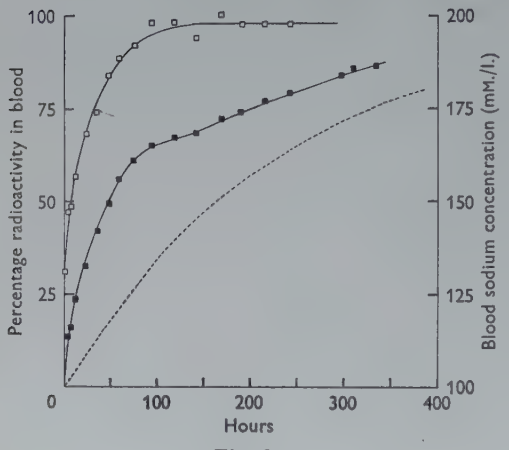


Fig. 6

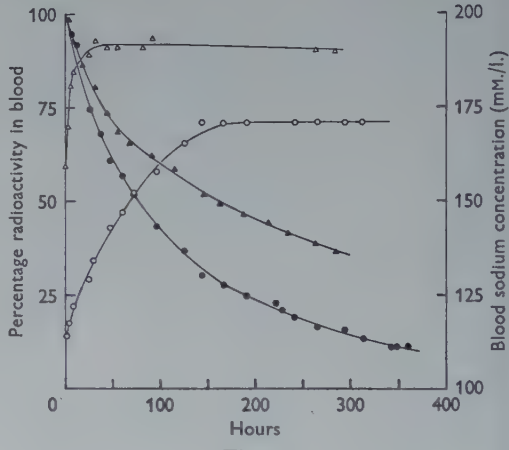


Fig. 7

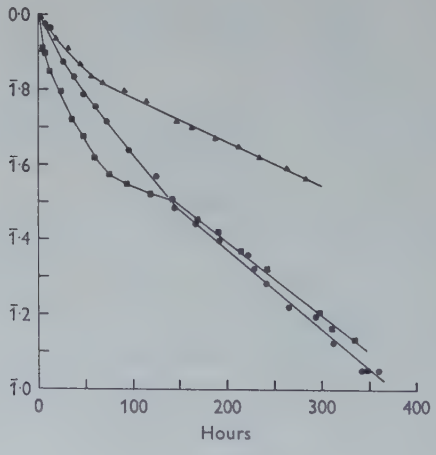


Fig. 8

Fig. 6. Uptake of $^{22}Na$ during net uptake of sodium by a depleted animal. ■, blood radioactivity; □, blood sodium concentration; – – –, shows the expected curve for uptake of $^{22}Na$ in the normal animal.

Fig. 7. Two experiments showing the loss of $^{22}Na$ during net uptake of sodium by a depleted animal. ●, ▲, blood radioactivity; ○, △, blood sodium concentration.

Fig. 8. ■, blood radioactivity from Fig. 6 plotted according to equation (iv); ●, ▲, blood radioactivity from Fig. 7 plotted according to equation (v).

$k_{out}$ found in the net uptake experiment when the animal had regained the steady state condition (i.e. from the slope of the straight part of the semi-logarithmic plot of the $^{22}Na$ results in Fig. 8). On comparing the initial rates of uptake of $^{22}Na$ in the normal and depleted animal, it was found that in this case uptake by the depleted animal was about 6·8 times the normal value.

### *The loss of* $^{22}Na$ *during the net uptake of sodium*

Experiments of the type described for the normal animal were used to study the loss of $^{22}Na$ from the blood during net uptake of sodium. The simultaneous changes of blood radioactivity and sodium concentration in two experiments are shown in Fig. 7. The $^{22}Na$ results are also plotted according to equation (v) in Fig. 8 (i.e. as a plot of $\log_{10}([Na^*_{in}]_t/[Na^*_{in}]_{t=0})$ against time). In both cases in Fig. 8 the steeper curved part of the line corresponds to the period of net uptake and the straight part to the steady state condition eventually reached. The value for $k_{out}$ found from the slope of the straight part of the line made it possible to construct the complete curve for loss of $^{22}Na$ in the normal animal. As in the previous section it was then possible to compare the initial stages of the calculated normal curve for each animal with the experimental curve given in Fig. 7. It was found that during the first part of the net uptake process $^{22}Na$ was lost at about 2–3 times the normal rate. In another experiment where the net uptake of sodium was particularly rapid the initial rate of loss of $^{22}Na$ exceeded the normal rate by as much as 6 times or even more. If the rate at which sodium is lost over the body surface depends on the concentration of the blood as in the case of passive outward diffusion, then the experiments show that the permeability of the body surface to $^{22}Na$ in the direction in to out is greater during the net uptake phase. If, however, the rate of loss of sodium were constant and not dependent on blood concentration, then the dilution of isotope caused by the net uptake of inactive sodium could give a result of the type which was observed. But the maximum initial rate of loss of $^{22}Na$ which could be expected under these conditions would be 1·5 times normal and not 2, 3 or even 6 times as was found. Thus during the net uptake process the permeability of the body surface to $^{22}Na$ in the direction in to out is greater than normal and is most marked in animals where net uptake of sodium is most rapid.

## THE SITE OF UPTAKE OF SODIUM

### *The uptake of sodium from the gill chambers*

The net uptake of sodium into the blood of a depleted animal was observed when artificial tap water was perfused through the gill chambers by the method of Berger & Bethe (1931). Aerated artificial tap water from a Krogh constant flow apparatus (Krogh, 1939) passed into a small glass T-piece which had two polythene jets of 0·5 mm. diameter opening posteriorly into the gill chambers. The animal was clamped in a 1 l. capacity tank fitted with a waste pipe and sealed with damp filter-paper to maintain a high humidity. The rate of flow of artificial tap water was about 400 ml./hr. (Lindroth, 1938). Sodium was gained rapidly by the

blood and a plot of $\log_{10}(1-(\text{Na gained})_t/(\text{Na gained})_{t=\infty})$ against time gave roughly a straight line which is designated by the crosses in Fig. 5. The slope of this line is sufficiently like those for totally immersed animals to indicate that most of the sodium gained must have been absorbed in the gill chambers.

*Silver staining of gills*

In view of claims that silver absorption is an active process, staining in the gills of normal animals was compared with that in animals killed with poisons. Normal animals were washed for about 1 hr. in distilled water and then placed in a 1 mM./l. solution of $AgNO_3$ in the dark. The animal died in about $\frac{1}{2}$ hr. After removing the branchiostegites the gills were washed thoroughly with distilled water and then removed and placed in a tube of distilled water. On exposing to sunlight for about 30 min. parts of the gills became brown. For further examination they were fixed in warm Bouin, dehydrated with alcohols, cleared in xylol and mounted in balsam. Other animals were placed in 2 mM./l. sodium azide solution, 3 mM./l. eserine and 0·1% potassium cyanide solutions. The animals appeared to be dead after 1 hr. in azide, 3 hr. in eserine and 2 hr. in cyanide. They were shown to be losing sodium after these times in azide and eserine. The dead animals were treated in the same way as the normal animals, except that the branchiostegites were removed prior to the first washing in distilled water and the $AgNO_3$ solution was stirred. Staining similar to that in normal animals was found to have occurred and in both cases it appeared to be confined to the cuticle. If the $AgNO_3$ solution used for an animal killed in cyanide contained 0·1% KCN no staining could be obtained. This was probably due to the formation of the soluble complex $K[Ag(CN)_2]$ instead of the precipitation of silver chloride in the gill and would possibly explain why Schmidt-Nielsen (1941) found no staining in the presence of cyanide. Silver staining is apparently a passive process in which silver ions diffusing inwards form a precipitate with the chloride ions diffusing outwards, as was found by Croghan (1958*b*) in *Artemia*. Even so, the technique gives useful information as to which parts of the gills are likely to be most permeable to ions.

The two main types of gills are the podobranch and arthrobranch. The podobranch has a flat basal plate from the dorsal border of which rises the stem bearing many branchial filaments. At its upper end the stem divides into two parts: the lamina, a corrugated plate folded upon itself, and the plume which is the free end of the stem and has branchial filaments. The arthrobranch has no basal plate or lamina but is otherwise similar. It was found that the brown staining was confined to the branchial filaments of the stem (except for the most basal) separating them sharply from the basal plate, stem, plume filaments and lamina. The only staining apparent in other regions was a scattering of dark-brown granules in the ends of some of the plume filaments and in the most basal of the stem branchial filaments. Thus the branchial filaments of the stem form an anatomically distinct region of the gill which is far more permeable to silver than the other regions.

### *The question of sodium uptake via the gut*

It was not found possible to block the mouth with any form of stopper without obviously affecting the animal. To find how much water was being taken into the gut animals were placed in tap water containing either phenol red or Evans's blue, both at concentrations which would be obvious in the narrowest part of the gut. After 24 hr. the gut was examined (in the presence of ammonia in the case of phenol red), but the only evidence of these dyes was a low dilution in the stomach. If tap water containing indian ink or carmine particles was used, compacted particles were found in the rectum after 24 hr. but were not obvious in the stomach. It would appear that if these fine particles can gain access to the gut some water must also enter. However, the dye experiments indicate that the amount of water must be a very small and in no sense a continuous drinking process. Maluf (1940) arrived at the same conclusion after examining the gut of animals which had been immersed in phenol-red solution.

## DISCUSSION

In distilled water the rate of loss of $^{22}Na$ was about 36% lower than in artificial tap water. Of this 36% about 5% was the result of a more dilute urine being produced. This indicates that the loss of $^{22}Na$ over the body surface into artificial tap water is not solely a process of passive diffusion. It is just possible that in distilled water some of the $^{22}Na$ being lost was regained before it was clear of the body surface, but the large volumes of distilled water which were used make this seem unlikely. A reduction in the rate of loss of $^{24}Na$ from *Artemia* placed in distilled water has been used as evidence of 'exchange diffusion' by Croghan (1958*b*). Exchange diffusion was proposed by Ussing (1947), Levi & Ussing (1948) to account for the fact that sodium fluxes as measured with isotope in frog sartorius muscle appeared to be higher than the amount of available energy would allow. They visualized a system in which particles capable of forming a sodium complex were oscillating thermally in a membrane between two compartments not in electrochemical equilibrium. When the particles came in contact with either compartment the combined ion could exchange for another one. By this means $^{22}Na$ in one compartment could exchange with inactive sodium in the other on a 1:1 basis theoretically without any work being performed and with no change in the concentration of either compartment. Values of permeability computed from results for penetration of $^{22}Na$ in this manner would be larger than those on which the actual tendency of the animal to gain or lose sodium depended. In an extreme case the membrane could be completely impermeable to net movements of sodium and yet $^{22}Na$ could be exchanged. By placing distilled water on one side of the membrane exchange diffusion would be prevented, and this suggests that the effect of distilled water on $^{22}Na$ outflux from the crayfish might be due to the removal of this component with the remaining losses being due to passive diffusion. It has been found that if the blood sodium concentration of *Astacus* is raised to a sufficiently high

level the uptake of $^{22}Na$ from artificial tap water practically ceases (Bryan, 1960*b*). As in these experiments there was sodium present inside and outside the animal there is no reason why, if it existed in the simple form described above, uptake of $^{22}Na$ by exchange diffusion should not take place unless its existence depends on the presence of active sodium transport. Thus the slower loss of $^{22}Na$ into distilled water may be a direct result of the prevention of active uptake of sodium. This is supported by the fact that in tap water containing only 0·02 mM./l. NaCl, outflux of $^{22}Na$ was considerably reduced in conjunction with reduced influx of sodium. Sodium concentrations as low as this are probably too low to saturate the uptake mechanism which appears to be saturated under steady state conditions in tap water containing 0·5 and 2·0 mM./l. NaCl. This agrees with the work of Shaw (1959) who found that the rate of uptake of sodium by *Astacus* was dependent on outside concentration mainly below 0·5 mM./l.

The process of net uptake of sodium by a depleted animal from artificial tap water as shown by $^{22}Na$ is the result of an increase in the influx of sodium opposed by an increased but smaller outflux. The initial high rates of influx and outflux decrease to the normal equal values in such a way that the rise in blood sodium concentration to a steady state condition is roughly exponential. This evidence also points to the presence of a form of $^{22}Na$ outflux linked to influx, in addition to diffusion losses, which becomes increasingly obvious when sodium transport is increased during net uptake.

It is possible to explain the results obtained by the following simple model. Consider a membrane separating a high internal sodium concentration from a low external concentration, so that active transport of sodium inwards is necessary to maintain the concentration difference. In the membrane is a rotating carrier which at every revolution brings in eight ions but not being 100% efficient causes the loss of two ions. If in the time taken for one revolution six ions were lost by passive diffusion then the system would be in a steady state. By placing distilled water outside the membrane, uptake by the carrier and the consequent leakage would cease and only six ions would be lost—a decrease in outflux of 25%. Similarly, a reduction in influx would also result in the leakage component of outflux being decreased. On the other hand, a sevenfold increase in the rate of rotation of the carrier, as might be found during net uptake of sodium, would in this model increase outflux by between two and three times, although passive diffusion losses would not be expected to alter to any great extent. The sodium pump may thus be acting as a type of exchange diffusion carrier in that there is a 1:1 exchange between some of the sodium entering by the pump and that lost by leakage. While under conditions of low sodium influx this exchange component may be negligible, under conditions of net uptake it becomes considerable and may greatly exceed losses by passive diffusion. In normal animals it probably accounts for about 30% of $^{22}Na$ movements. A similar conclusion was reached by Shaw (1959) who found that under conditions fairly similar to those of the present work, influx of $^{24}Na$ exceeded the actual tendency for the animal to gain sodium by about 20% due to what might be a form of exchange diffusion. As theoretically the 1:1 exchange need not involve the

performance of work, only 70% of influx as measured with $^{22}Na$ in the normal animal can definitely be regarded as active transport.

Although there was no evidence of any control of sodium balance by the eyestalks a few observations indicated that hormonal control might be present. In two cases where net uptake of sodium by the blood was observed the sodium appeared to overshoot the steady state level reached eventually. Also, in the uptake of $^{22}Na$ during net gain of sodium (Figs. 6, 8) there were indications that just prior to reaching the steady-state blood sodium concentration the uptake of $^{22}Na$ actually fell below the normal rate. This was found in two experiments. Overcompensation of this type might be expected if sodium balance were hormonally controlled.

Net uptake of sodium from the perfused gill chambers showed that almost certainly the gills are responsible for most of the sodium influx. As it was found that the crayfish does not drink very much of the medium, the gills probably take up all the sodium absorbed by starved animals. The evidence from silver-staining experiments indicated that the cuticle of the stem branchial filaments is far more permeable to silver ions than the rest of the gill. It is quite possible that this region is more permeable to other ions also and could be the main site of the ion uptake mechanisms and much of the outflux over the body surface. Pütter (1911) estimated that the gill area of a 20 g. crayfish was 60 cm.$^2$ and this would have enabled the movement of sodium across the gills to be calculated in terms of area for comparison with other animals. The finding of differences within the gills makes this impossible at present.

## SUMMARY

1. In distilled water or artificial tap water with a very low sodium concentration, sodium uptake by *Astacus* is prevented or reduced and $^{22}Na$ outflux is subnormal. This is accounted for to only a small extent by reduced renal sodium losses.

2. Sodium-depleted animals replaced in artificial tap water regain sodium in a roughly exponential manner. This is shown by $^{22}Na$ to be the result of a considerable increase in sodium influx coupled with an increased but lower outflux.

3. Sodium outflux appears to consist of three components: urine losses, passive diffusion losses over the body surface and what may be an 'exchange diffusion' component which is high during high influx and minimal in distilled water. This latter component represents about 30% of sodium exchange under normal conditions.

4. Eyestalk removal did not affect the ability of *Astacus* to absorb sodium.

5. In starved animals the gills take up most of the sodium absorbed and the gut is relatively unimportant.

6. Silver staining of the gills is a passive process and the cuticle of the branchial filaments of the gill stem is selectively stained. This region would be a suitable site for ion uptake mechanisms.

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# SODIUM REGULATION IN THE CRAYFISH *ASTACUS FLUVIATILIS*

## III. EXPERIMENTS WITH NaCl-LOADED ANIMALS

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## INTRODUCTION

In a previous paper (Bryan, 1960*b*) the effect of subnormal blood concentration on the movements of sodium between the blood and medium was studied in *Astacus*. The effect of increasing blood sodium concentration above the normal level has also been studied. An increase in the blood chloride and sodium concentration of crayfish placed in diluted sea water has been demonstrated in experiments by Berger (1931) and Bogucki (1934). In most of the experiments to be described animals were loaded with sodium by leaving them in artificial tap water having a high NaCl concentration. The net uptake of sodium under the conditions found in this type of experiment has been studied with the aid of $^{22}Na$ and also the effect of increased blood sodium concentration on that of the tissues. The way in which sodium is removed from these loaded animals placed in normal artificial tap water has also been investigated. It had been indicated previously (Bryan, 1960*a*) that heavily fed animals which might have gained extra sodium in this way had a urine sodium concentration which in some cases was several times the level found after a few days' starvation. This role of the excretory organs in sodium removal has been studied further and $^{22}Na$ has been used in the determination of corresponding sodium movements across the body surface (gills).

Most of the methods used in these experiments were described by Bryan (1960*a*) and modifications only are given. The term 'normal animal' refers to crayfish under steady state conditions in normal artificial tap water which contains 2 mM./l. NaCl. Except where otherwise stated experiments were performed at 20° C.

## EXPERIMENTS IN HIGH EXTERNAL SODIUM CONCENTRATIONS

### *The effect of high external sodium concentrations on blood and urine*

Animals were placed for up to 8 days in artificial tap water containing increased amounts of NaCl. If the external sodium concentration was to be greater than 250 mM./l., the animal was first kept for 2 days in a 200 mM./l. solution. Experiments showed that in a solution containing 200 mM./l. NaCl the blood sodium concentration increased and equilibrium was reached in less than 4 days, while in

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250 mM./l. solution this time was increased to 6 days. After preadaptation to a 200 mM./l. solution animals could survive for more than 2 weeks in a 300 mM./l. solution and up to a week in 400 mM./l. It is quite probable that animals placed in concentrations greater than 300 mM./l. did not reach complete equilibrium as the time for its attainment was likely to be longer than the 5–6 days allowed. After this length of time the animals usually became moribund. Room temperature was used for these experiments and varied between 16 and 20° C. The general effect of raising the external sodium concentration was that both blood and urine concentrations increased (Fig. 1). This increase was not very marked until the external sodium concentration exceeded 100 mM./l. At higher external concentrations effects on the blood and urine were more obvious and their sodium concentrations tended towards equality with that of the medium. It seems likely that under these extreme conditions active movements of sodium effected by the gills or excretory organs had ceased.

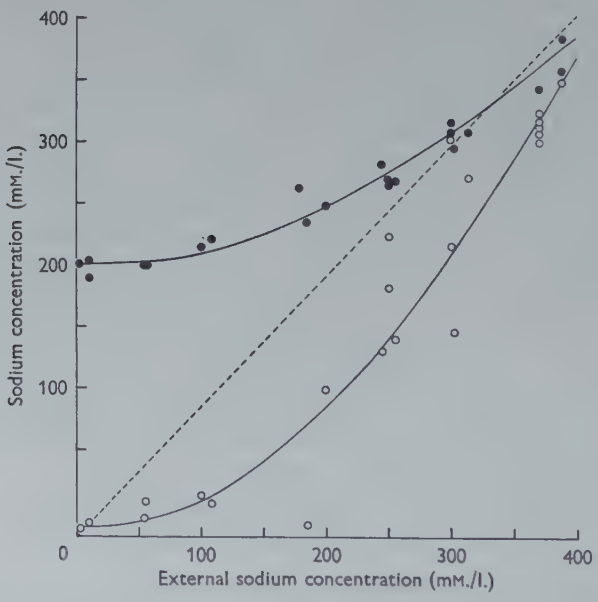


Fig. 1. Effect of increasing the sodium concentration of artificial tap water on that of the blood and urine. ●, blood sodium concentration; O, urine sodium concentration.

### *The loss of* $^{22}$Na

#### (*a*) *In artificial tap water containing* 10 mM./l. NaCl

The results of two experiments, which are shown in Fig. 2 plotted according to equation (v) (Bryan, 1960*a*), indicate that $^{22}$Na was lost at about the same rate in both media although rather more must have been lost as urine in the 10 mM./l. solution. As the blood sodium concentration was not appreciably different in the two solutions then presumably the rate of uptake of sodium remained constant.

(*b*) *In artificial tap water containing* 245 mM./l. NaCl

Herrmann (1931) showed that in sea water isotonic with the blood there was initially no production of urine by the animal. Therefore, in isotonic solution the initial loss of $^{22}Na$ may probably be attributed to the body surface only. After observing the rate of loss of $^{22}Na$ into normal artificial tap water the animal was placed in artificial tap water containing 245 mM./l. NaCl which was isotonic with the blood and the changes in blood sodium concentration, blood activity and urine sodium concentration determined (Fig. 3).

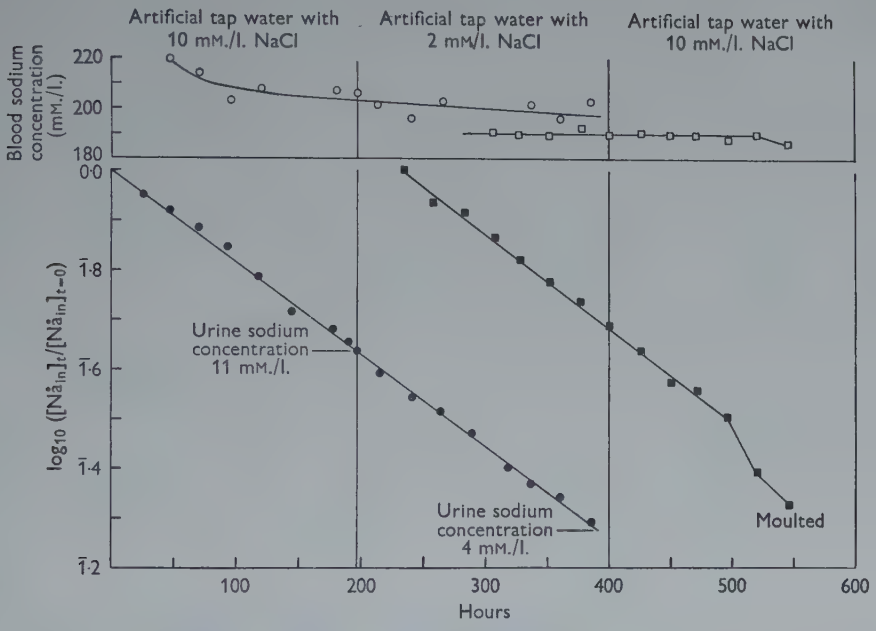


Fig. 2. Two experiments comparing the loss of $^{22}Na$ into artificial tap water containing 2 mM./l. NaCl with that into artificial tap water containing 10mM./l. NaCl. ●, ■, blood radioactivity plotted according to equation (v); ○, □, blood sodium concentration.

The net gain of sodium by the blood in the isotonic solution was accompanied by, in the early stages, a fourfold increase in the rate of loss of $^{22}Na$. At the same time the urine sodium concentration rose rapidly, after a period of delay, to a level many times normal. This delay was presumably due to so little urine being produced at first that the concentration of the dilute urine already in the bladder was not appreciably increased. When the blood sodium concentration had reached the new steady state condition the rate of loss of $^{22}Na$ as found from the slope of the semi-logarithmic plot in Fig. 3 had returned to a figure rather higher than that for the normal animal (Table 1). An otherwise identical experiment performed at an external concentration of 200 mM./l. NaCl gave very similar results and values for $k_{out}$ under the steady-state conditions are also shown in Table 1. These figures for $k_{out}$ include $^{22}Na$ losses via the urine. The volume of urine produced under the

high sodium conditions is not known, but at such a high sodium concentration only a small amount need be produced for renal sodium losses to exceed those of a normal animal. If this were the case, then the rate of loss of $^{22}Na$ over the body surface might be very similar under the two sets of conditions.

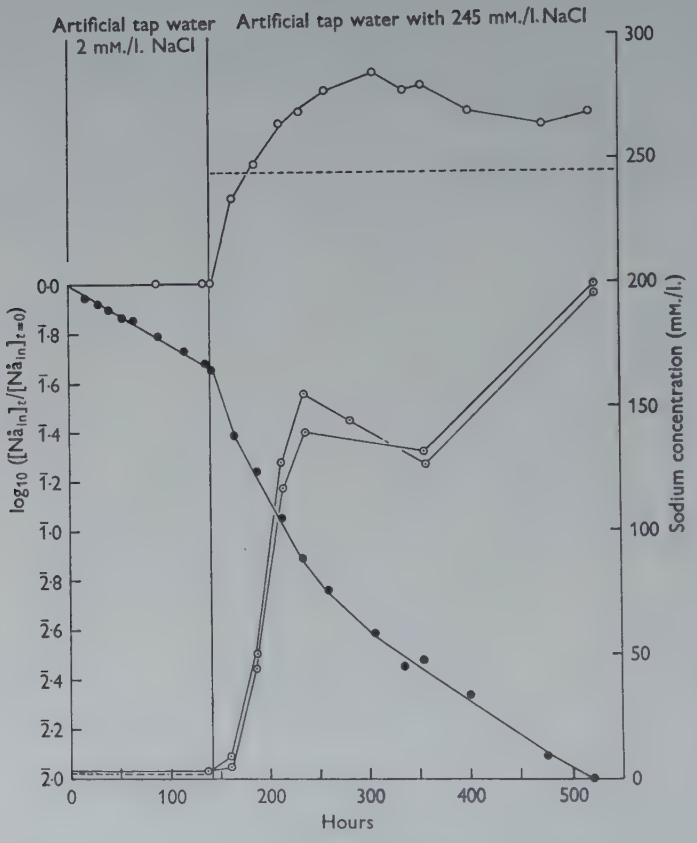


Fig. 3. Effect of raising the sodium concentration of artificial tap water from 2 mM./l. to 245 mM./l. ●, blood radioactivity plotted according to equation (v); ○, blood sodium concentration; ⊙, urine sodium concentration.

Table 1. *Values of* $k_{out}$ *for the loss of* $^{22}Na$ *under steady state conditions in high and low external* NaCl *concentrations*

| External sodium concentration (mM./l.) | Blood sodium concentration (mM./l.) | $k_{out}$ hr.$^{-1}$ |
|---|---|---|
| 2 | 200 | 0·00386 |
| 200 | 246·5 | 0·00445 |
| 2 | 200 | 0·00537 |
| 245 | 280 | 0·00633 |

## THE EFFECT OF BLOOD SODIUM CONCENTRATION ON THAT OF THE TISSUES

It has been shown previously, using $^{22}Na$, that about 38% of the exchangeable sodium in the crayfish appears to lie in the tissues (Bryan, 1960*a*). Whether this percentage is the same at all blood sodium concentrations depends on the ability of the tissues to regulate the intracellular sodium concentration. The particular tissue examined was the muscle found in the abdomen (extensors and flexors of the abdomen). Shaw (1955*a*) showed that in *Carcinus* the ionic composition of single muscle fibres was nearly the same as that of a whole muscle which had been washed for less than 30 sec. In the crayfish large easily separated muscle fibres similar to those of *Carcinus* are found, and owing to the lower salt content of the blood the chances of contamination of the tissue should be less. Whole muscles were therefore used to find the effect of blood sodium concentration on muscle sodium and potassium levels.

Subnormal blood sodium concentrations were achieved by exposing animals to distilled water which was changed frequently. High blood sodium concentrations were obtained in the way described in the previous section. Prior to tissue analysis blood samples were taken for sodium and potassium estimation. The abdominal muscle was then dissected out, washed for about 20 sec. in distilled water and dried with filter-paper. After weighing in a 100 ml. conical flask the tissue was dried to constant weight to find the water content. Organic material was removed from the sample by evaporating off each of about five 5 ml. additions of 50% redistilled nitric acid over an electric fire element. The residue was dissolved in hot distilled water and diluted for sodium and potassium estimation. Samples were normally wet-ashed in groups of four or five together and a blank flask enabled a correction to be applied for any contamination by sodium or potassium from the acid. This was extremely small. It was found that if the muscle was cut into two pieces, the values obtained for each never differed by more than 4%.

Sodium and potassium concentrations for abdominal muscle and the potassium concentrations of the blood are shown plotted against blood sodium concentration in Fig. 4. Although at the higher blood sodium concentrations there is a nearly linear relationship with the muscle sodium concentration it is not one of simple proportion as was found in *Carcinus* by Shaw (1955*b*). The ratio $[Na_{out}]/[Na_{in}]$ for the muscle is relatively constant at subnormal blood sodium concentrations, but at high concentrations is reduced by up to 50% (Table 2). Thus at high blood concentrations relatively more sodium comes to lie in the tissue. As a result the value of the sodium space $V'$ (Bryan, 1960*a*) will be increased. This has a bearing on the results of experiments to be considered later. Potassium balance was not markedly affected by the changes in sodium concentration. The mean value for the blood potassium concentration was 4·7 mM./l. and that for the muscle 97 mM./kg. $H_2O$.

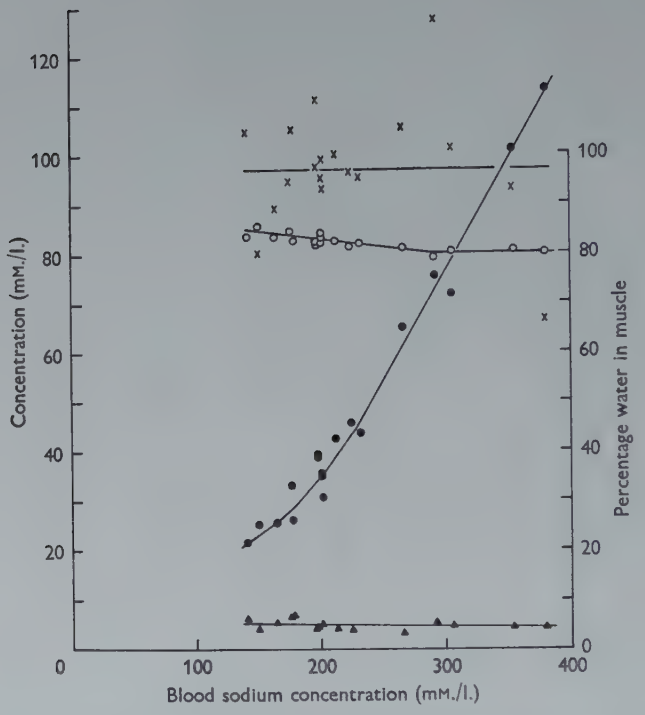


Fig. 4. Effect of blood sodium concentration on muscle. ●, muscle sodium concentration; ○, muscle water content; ×, muscle potassium concentration; ▲, blood potassium concentration.

Table 2. *Variation of muscle sodium concentration with blood sodium concentration*

| Blood sodium concentration $[Na_{out}]$ (mM./l.) | Muscle sodium concentration $[Na_{in}]$ (mM./kg. $H_2O$) | $\frac{[Na_{out}]}{[Na_{in}]}$ |
|---|---|---|
| 141 | 21·6 | 6·53 |
| 150 | 25·5 | 5·89 |
| 164 | 25·4 | 6·47 |
| 177 | 33·0 | 5·37 |
| 179 | 26·2 | 6·83 |
| 198 | 38·8 | 5·11 |
| 198 | 39·1 | 5·07 |
| 201 | 30·5 | 6·59 |
| 201 | 34·8 | 5·78 |
| 201 | 35·2 | 5·71 |
| 213 | 42·4 | 5·02 |
| 225 | 45·7 | 4·93 |
| 233 | 43·6 | 5·34 |
| 266 | 65·0 | 4·10 |
| 292 | 75·5 | 3·87 |
| 306 | 71·8 | 4·26 |
| 354 | 102·0 | 3·47 |
| 380 | 113·0 | 3·36 |

## THE REMOVAL OF EXCESS SODIUM

### *The loss of sodium from* NaCl-*loaded animals in normal artificial tap water*

Animals preadapted to artificial tap water containing 200 mM./l. NaCl were placed in a 430 mM./l. solution for 4 days. After loading with NaCl in this way they were clamped in a tank of normal artificial tap water and simultaneous determinations of blood and urine sodium concentrations made until there was no further change. A typical result is shown in Fig. 5.

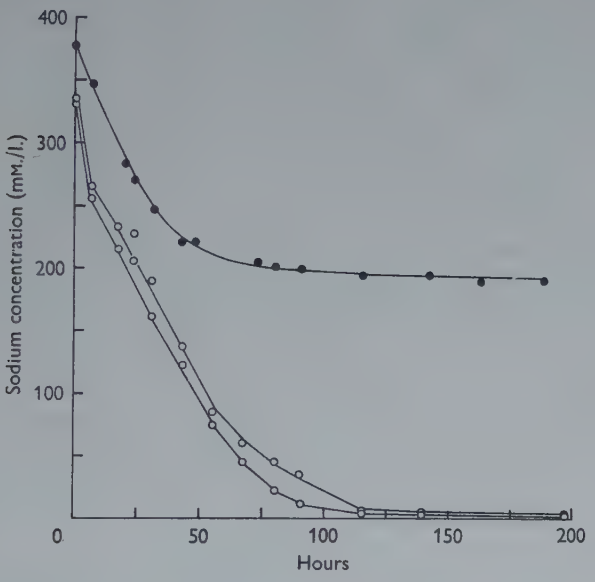


Fig. 5. Loss of sodium from an NaCl-loaded animal in artificial tap water. ●, blood sodium concentration; ○, sodium concentration of urine from both excretory organs.

The curve for net loss of sodium from the blood is exponential in character. Corresponding with this curve are those for urine sodium concentration. The high urine sodium concentrations recorded initially indicate that renal losses must contribute largely to net sodium loss. Further confirmation comes from a few experiments in which the volume of urine produced by NaCl-loaded animals was measured and found to be greater than that produced after unloading (Table 3).

Table 3. *Volumes of urine produced by* Astacus *in artificial tap water under* NaCl-*loaded and normal conditions after unloading*

| Approximate blood sodium concentration (mM./l.) | Volume of urine produced (% weight increase/24 hr.) | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | Mean |
| 340 | 8·6 | 8·8 | 12·1 | 8·9 | 10·0 | 9·7 |
| 190 | 6·2 | 6·5 | 9·3 | — | — | — |

To confirm that blood sodium concentration changes were not dependent to any extent on changes in body volume, animals in which the blood had been raised to a sodium concentration of about 340 mM./l. were weighed at intervals in artificial tap water. During the first 24 hr. of net sodium loss the body weight fell by 3% but subsequent losses were negligible.

*Uptake of $^{22}$Na during net loss of sodium*

The uptake of $^{22}$Na during the net loss of sodium by NaCl-loaded animals was measured in the way described for normal animals (Bryan, 1960*a*). In this case, as well as maintaining a constant level of activity in the artificial tap water, it was necessary to keep the external NaCl concentration constant at 2 mM./l. Any increase in external sodium concentration was corrected by replacing some of the medium with a solution of the same activity not containing sodium. In four experiments animals were used which had been loaded by a few days' exposure to artificial tap water containing 375 mM./l. NaCl. To check that the results were not due to the lengthy period in this solution two 25 g. animals were used which had been loaded by injection. About 0·15 ml. of a 5 M./l. solution of NaCl in distilled water was injected over a period of about 40 min. into the pericardium of the clamped animal using an 'Agla' micrometer syringe. The results of three experiments are shown in Fig. 6. While sodium was being lost from the blood $^{22}$Na uptake was curtailed and became normal as the blood sodium concentration approached a constant level. Curtailment of $^{22}$Na uptake was most apparent in two animals (one shown in Fig. 6) which had initial blood sodium concentrations in excess of 300 mM./l. In these animals, even after 20 hr. when a considerable net loss of sodium had taken place, the level of $^{22}$Na in the blood was only about 5% of that expected in a normal animal. It seems reasonable to suppose that during the first few hours $^{22}$Na uptake was even more limited. Reduced $^{22}$Na uptake was apparent in the injected animals and Fig. 6 also shows that a high urine sodium concentration was recorded about 2 hr. after the injection.

*Loss of sodium over the body surface during curtailed uptake*

At blood sodium concentrations higher than 300 mM./l. sodium uptake from artificial tap water was nearly reduced to zero. Under these conditions the net loss of sodium would almost equal the total sodium outflux. To measure the rate of net loss over the body surface only, the excretory pores were blocked with dental cement. This could be done for from 5 to 10 hr. without damaging the animal. Crayfish weighing about 10 g. loaded with NaCl and with the excretory pores blocked were each placed in 100 ml. of aerated artificial tap water in a beaker and the increase in the sodium concentration of the medium observed for about 5 hr. The pores were then unblocked over the beaker and further samples of medium were taken for analysis until no more sodium was gained (Fig. 7). While sodium was being gained the medium was changed at intervals so that the concentration was never too far removed from 2 mM./l. The initial high blood sodium concentration was taken as the mean value for other animals exposed to the same external

concentration for the same length of time. The final blood sodium concentration was measured when no more sodium was gained by the medium. It has been assumed that the total net fall in blood sodium concentration (in mM./l. blood) is equivalent to the gain of sodium by the medium (in μequiv./g. animal). Thus the rate of loss when the excretory pores were blocked could be expressed as mM./l. blood/hr. and compared with the rate of sodium loss in the normal animal which was found later by means of a $^{22}Na$ uptake experiment. The assumption may not

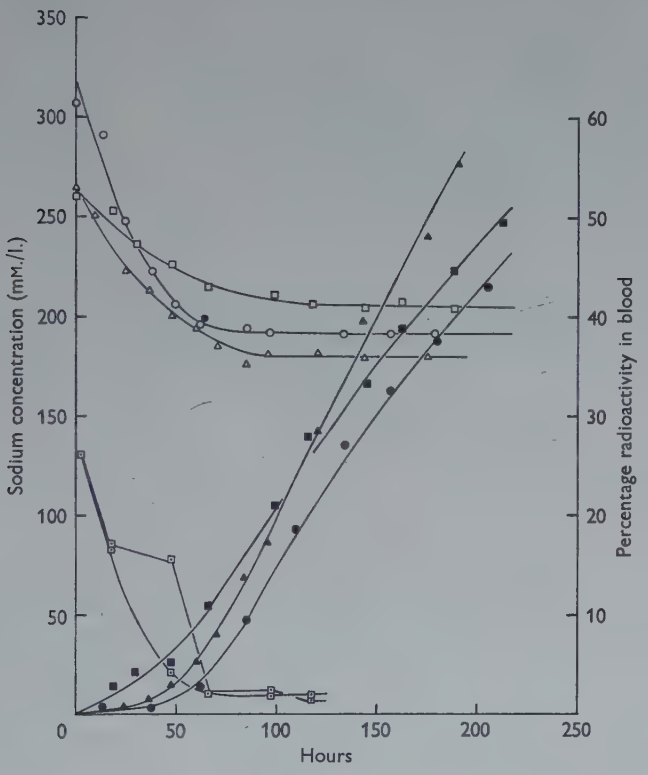


Fig. 6. Three experiments showing the uptake of $^{22}Na$ from artificial tap water during the net loss of sodium from an NaCl-loaded animal. The animal denoted by square symbols was loaded by injection. ●, ▲, ■, blood radioactivity; ○, △, □, blood sodium concentration; ⊡, sodium concentration of urine from both excretory organs of the injected animal.

be strictly true, as it has been shown in a previous section that in NaCl-loaded animals the abdominal muscle contains a greater percentage of the body sodium than normal. This would make the total loss in μequiv./g. animal rather higher than it would otherwise be. As a result the rate of loss of sodium with the excretory pores blocked expressed as mM./l. blood/hr. would be rather too low. The results with corrections applied are shown in Table 4.

The rate of loss of sodium over the body surface is greater when the animal is in the NaCl-loaded condition. This would be expected if losses were due to passive

outward diffusion, when the rate of loss would be equal to $[Na_{in}]k'$, where $[Na_{in}]$ is the blood sodium concentration and $k'$ the permeability constant of the crayfish body surface. Values for $k'$ found in these experiments are shown in Table 5.

Table 4. *The rate of loss of sodium over the body surface into artificial tap water from* NaCl-*loaded animals during the initial phase of net loss (when uptake was curtailed) compared with that found when a steady state was eventually reached*

| | Animal 1 | Animal 2 |
|---|---|---|
| Initial high blood sodium concentration (mM./l.) | 310 | 310 |
| Initial rate of net loss of sodium over the body surface when excretory pores blocked (mM./l. blood/hr.) | 1·55 | 2·35 |
| Normal blood sodium concentration $[Na_{in}]$ (i.e. when steady state achieved after net loss of sodium (mM./l.)) | 186 | 180 |
| $k_{out}$ hr.$^{-1}$ (from $^{22}$Na uptake experiment) | 0·00494 | 0·00745 |
| Rate of loss of sodium $[Na_{in}]$ $k_{out}$ (mM./l. blood/hr.) | 0·92 | 1·34 |
| Correction for losses in urine (mM./l. blood/hr.) | 0·04 | 0·04 |
| Rate of loss of sodium over the body surface (mM./l. blood/hr.) | 0·88 | 1·30 |

Table 5. *Permeability constants for the loss of sodium over the body surface calculated from the results in Table* 4

| $[Na_{in}]$ (mM./l.) | Rate of loss of sodium (mM./l. blood/hr.) | Permeability constant $k'$ |
|---|---|---|
| 186 | 0·88 | 0·0047 |
| 310 | 1·55 | 0·0050 |
| 180 | 1·30 | 0·0072 |
| 310 | 2·35 | 0·0076 |

The constant is rather higher under NaCl-loaded conditions and would probably be further increased if the greater percentage of body sodium in muscle could be taken into account. Also, previous work (Bryan, 1960*b*) has indicated that under normal conditions outflux of sodium as measured by $^{22}$Na includes a very variable exchange component in addition to losses by diffusion. This component, if present, would make the normal values of $k'$, given above, too high. It would seem that under NaCl-loaded conditions the permeability of the body surface to sodium in the direction in to out is rather higher than that of the normal animal. However, there is always the possibility that the blockage of the excretory pores was not 100% perfect and it might be unwise to regard this permeability difference as being very significant.

*Loss of sodium via the excretory organs during curtailed uptake*

If an NaCl-loaded animal was placed in a small volume (100–300 ml.) of artificial tap water and the sodium concentration of this solution was determined until there was no further change, then the amount gained by the medium (or lost by the animal) plotted against time gave an exponential curve. This was fairly closely governed by an equation of a form such that a plot of $\log_{10}(1-(\text{Na lost})_t/(\text{Na lost})_{t=\infty})$ against

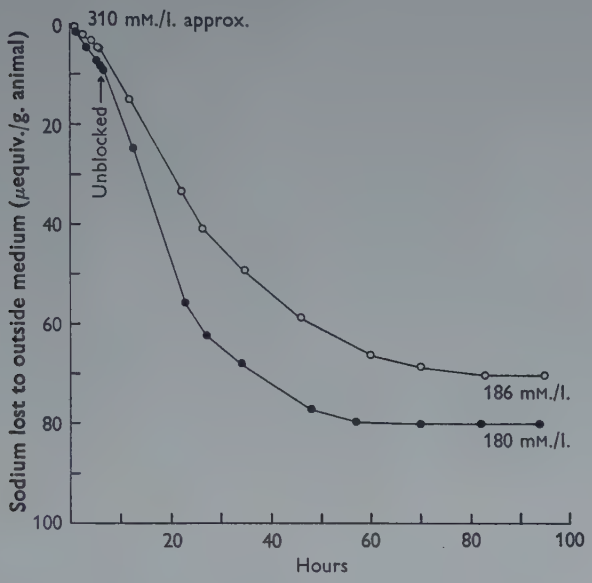


Fig. 7. Two experiments showing the net loss of sodium into artificial tap water from NaCl-loaded animals with excretory pores blocked for the first 6 hr. The figures at the beginning and end of each curve give the corresponding blood sodium concentrations.

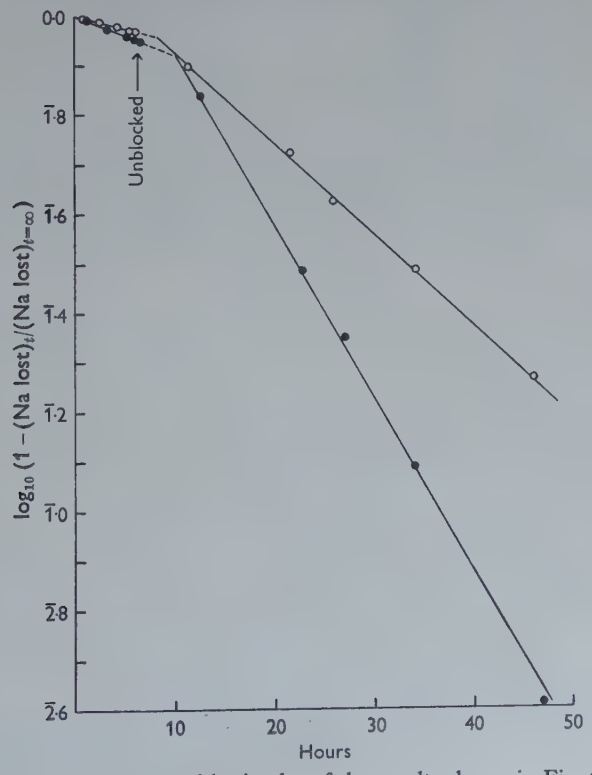


Fig. 8. A semi-logarithmic plot of the results shown in Fig. 7.

time gave a straight line. $(\text{Na lost})_t$ is the amount of sodium in $\mu$equiv./g. lost by the animal after time $t$ and $(\text{Na lost})_{t=\infty}$ is the total net loss of sodium. During these experiments the outside solution was changed at intervals so that the sodium concentration did not become too far removed from 2 mM./l. The rate of net loss of sodium via the body surface was compared with the total rate of loss in animals loaded with NaCl to an extent where sodium influx was negligible. The procedure of the previous section was again used to distinguish loss over the body surface from that via the excretory organs (Fig. 7). A plot was then made of $\log_{10}(1-(\text{Na lost})_t/(\text{Na lost})_{t=\infty})$ against time as shown in Fig. 8. From the slopes of the straight lines obtained when the excretory pores were blocked and unblocked, it was possible to compare the total rate of net loss with that through the body surface during the initial period when sodium influx was extremely small. Table 6 shows the percentage of net sodium loss for which the excretory organs were responsible during the first few hours of sodium removal.

Table 6. *Sodium losses via the excretory organs of* NaCl-*loaded animals during the first few hours of net loss into artificial tap water*

| Initial blood sodium concentration (mM./l.) | Initial sodium losses via the excretory organs (% of total net sodium loss) |
|---|---|
| 310 | 69·6 |
| 310 | 73·8 |
| 310 | 73·5 |
| 340 | 65·6 |
| 340 | 68·2 |
| 285 | 74·0 |

These results contrast sharply with those for the normal animal where renal losses constituted only about 6% of total sodium outflux as measured with $^{22}$Na (Bryan, 1960*a*).

## DISCUSSION

The crayfish was able to maintain a fairly normal blood sodium concentration in artificial tap water containing up to 100 mM./l. NaCl. It was found that influx and outflux of sodium were practically the same in normal artificial tap water and that containing 10 mM./l. NaCl. Where the external sodium concentration approached that of the blood or exceeded it, thus reducing diffusion losses and urine production considerably, a net increase in blood sodium concentration to a new steady state was found. Like net uptake by depleted animals (Bryan, 1960*b*), the process involved a considerably increased outflux of $^{22}$Na over the body surface in the initial stages. This returned to a fairly normal value when the new steady state was reached. The concentration difference maintained between blood and medium under these new conditions was small and became smaller as the external concentration was increased above the normal blood sodium concentration of 200 mM./l. Similarly, the concentration difference between the blood and urine became progressively smaller. It seems likely that as the steady-state blood sodium concentra-

tion is increased so active sodium movements are decreased. At external concentrations in the region of 400 mM./l. a steady state was reached where the concentrations of blood, urine and medium tended to be equal. Under these extreme conditions active sodium movements had probably ceased. In support of this is the finding that in normal artificial tap water, sodium influx was curtailed almost completely if the blood sodium concentration exceeded 300 mM./l. At lower concentrations influx was curtailed to a lesser degree.

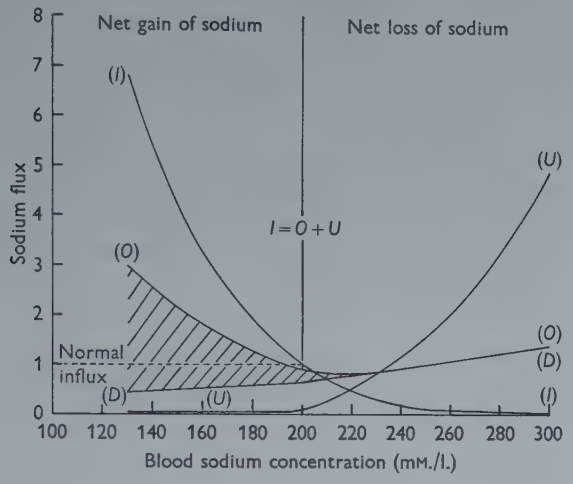


Fig. 9. Diagram showing the changes in sodium flux with blood sodium concentration during the net uptake and net loss of sodium from *Astacus* in artificial tap water containing 2 mM./l. NaCl. (*I*) is influx measured with $^{22}Na$. (*O*) is total outflux over the body surface (measured with $^{22}Na$ during net uptake and in the normal animal). (*D*) is the possible extent of diffusion losses over the body surface. (*U*) is sodium losses in the urine. Cross-hatching denotes the possible extent of the exchange component of outflux.

Muscle sodium concentration rose with increasing blood concentration, but the relationship was not one of simple proportion. At high blood sodium concentrations there was relatively more sodium in the muscle than normal. A similar result was obtained in the fresh-water crab *Potamon niloticus* by Shaw (1959). He considers that exchangeable muscle sodium is probably present in the outer regions of the fibre under normal conditions, but that at high blood concentrations some sodium also penetrates into the fibre interior. When animals loaded with NaCl were replaced in artificial tap water the blood sodium concentration fell exponentially and this involved curtailment of influx combined with increased urine production (due to the higher blood osmotic pressure) at a high sodium concentration. Thus in an animal with a blood sodium concentration of 300 mM./l. influx was reduced nearly to zero, loss over the body surface was increased roughly in proportion to the higher blood concentration and the excretory organs lost sodium initially to the extent of 70% of the total outflux.

Some of the results presented in this paper and in previous papers (Bryan, 1960*a*, *b*) are summarized in Fig. 9. This diagram shows the changes in sodium

flux which occurred during either the net uptake or net loss of sodium from *Astacus* in artificial tap water containing 2 mM./l. NaCl. The influx of sodium ($I$) measured with $^{22}Na$ is given the value of 1·0 in the normal animal where it balances outflux at a blood sodium concentration of 200 mM./l. Outflux consists of 0·06 urine losses ($U$) and 0·94 outflux over the body surface ($O$). This latter component appears to be further divisible into 0·63 which is probably passive diffusion ($D$) and 0·31 which disappears when $^{22}Na$ outflux is measured in distilled water ($O-D$). It is presumably this smaller fraction of outflux over the body surface in the normal crayfish which is reduced when influx falls in artificial tap water having a very low sodium concentration and which is increased when influx rises during net uptake of sodium. It has been suggested previously (Bryan, 1960*b*) that this apparent linkage between a component of outflux and influx is caused by a back leakage of the sodium uptake mechanism. This leakage creates a situation which might be described as influx-dependent 'exchange diffusion'. The possible extent of this exchange component is shown by the cross-hatching in Fig. 9. During net uptake of sodium by the depleted animal $^{22}Na$ influx ($I$) was initially about seven times normal, urine losses were about one-eighth normal and the exchange component was considerable. The reverse condition was found during net loss. Influx at a blood sodium concentration in excess of 300 mM./l. was negligible, urine losses initially represented up to 70% of the total outflux and there was no exchange component. There was also some unconfirmed evidence that the permeability of the body surface to net sodium loss was rather higher than normal under these conditions.

The diagram in Fig. 9 as a whole, indicates that there is a close connexion between $^{22}Na$ influx and blood sodium concentration. A similar sort of relationship also seems to hold for losses in the urine. It is thought likely that in the excretory organ the walls of the nephridial canal reabsorb sodium from the primary isotonic urine produced in the coelomic sac. If this absorption mechanism is built on the same principle as that in the gills the rate of reabsorption of sodium would be closely related to blood sodium concentration. At high concentrations reabsorption would be curtailed and result in the production of a high urine concentration. Thus when the blood sodium concentration was raised to nearly 400 mM./l. and the animal placed in artificial tap water, influx through the gills was very low and the urine sodium concentration was initially nearly equal to that of the blood. Under conditions of sodium depletion, influx through the gills was high and a corresponding rapid absorption in the nephridial canal would explain the reduction in urine sodium concentration which was found. The minimum sodium concentration to which the urine could be lowered was rather less than 1 mM./l. Assuming that this theory is correct, the rates of uptake of sodium by the gills and nephridial canal will always vary in exact relation to each other depending on the blood sodium concentration. If outflux through the body surface is due to diffusion losses plus the component linked to influx, one then has four different processes whose rates are bound to each other by virtue of direct or indirect dependence on the blood sodium concentration. As the rates of these processes vary in different ways there

is only one blood sodium concentration at which they can balance, i.e. where influx = outflux over body surface + losses in urine. This is shown as 200 mM./l. in Fig. 9. Any increase in blood sodium concentration would cause influx to be reduced and urine losses to be increased thus resulting in a fall of concentration. As it fell the rates of influx and reabsorption in the excretory organs would automatically increase and so prevent further loss. It is possible, therefore, that blood sodium concentration oscillates about a mean normal value. This theory thus invokes control of sodium balance by a single type of mechanism operating under the general control of the blood concentration in two opposed organs. The rates of the active processes will be dependent on the energy supplied to them. It may be that the changing blood sodium concentration directly affects the rate of a reaction leading to the production of this energy. On the other hand, control might be mediated by a metabolic hormone whose rate of secretion depends on the blood salt concentration. The possibility of some form of hormonal control by the eyestalk was examined (Bryan, 1960*b*) but no definite evidence of this could be found. However, other likely endocrine regions were not tested.

## SUMMARY

1. In external sodium concentrations of up to 100 mM./l. the blood sodium concentration of *Astacus* is only slightly increased. As the external level approaches or exceeds the normal blood sodium concentration of 200 mM./l. so the increase becomes more marked. Similarly, there is an increase in urine sodium concentration. This net gain of sodium is accompanied by a considerable rise in sodium outflux as shown by $^{22}Na$. At external concentrations exceeding 300 mM./l., blood and urine concentrations rise to a similar level and active sodium movements appear to cease.

2. With increased blood sodium concentration the level in the muscles rises also. This relationship is not one of simple proportion and at high concentrations there is relatively more sodium in the muscles.

3. In artificial tap water animals with a high blood concentration lose sodium until the normal level is regained. This net loss is due to influx being much lower and outflux much higher than normal. Of the outflux, up to 70% is initially due to renal losses and losses over the body surface are higher than normal due to the excess sodium in the blood.

4. From the results given in this and previous papers the way in which sodium balance may be achieved under normal conditions is discussed.

Again I should like to thank Dr J. A. Kitching, under whose supervision this work was carried out, for his advice and helpful criticism, and also Prof. J. E. Harris, F.R.S., for his interest in the problem. I am indebted to the Department of Scientific and Industrial Research for a maintenance grant.

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# SPEED AND STAMINA IN THREE FISH

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## INTRODUCTION

The speeds already reported (Bainbridge, 1958*a*) as attainable by various fish after measurement in the 'fish wheel' were calculated from ciné film records of swimming over a minimum distance of 1 m. Such figures give little indication of the length of time for which a particular speed can be sustained. The 29·3 cm. trout reported as travelling at 320 cm./sec., for example, had to swim at this speed for only $\frac{1}{3}$ sec. in order to qualify for inclusion; while the lowest speeds reported, of the order of 10 cm./sec., required only 10 sec. of swimming.

A variety of reasons make it desirable to know how long particular speeds can be sustained by fish of different sizes. The most efficient design of fish-passes, for example, particularly those demanding periods of violent activity separated by periods of rest, could be dependent upon parameters of this kind. The speed at which trawls and other fishing devices should be manipulated, and the precise design of the net opening and the relative positions and lengths of headline, ground-rope and tickler-chain could also profitably be examined in the light of such information. Finally, a proper assessment of the physiological problems associated with swimming and the relationship between power output, drag and speed and a consideration of the degree of correspondence between fatigue in fish and other animals, are all dependent upon a precise knowledge of the ability of fish to sustain particular speeds of swimming.

It has already been briefly reported (Bainbridge, 1958*b*) that the duration of a burst of swimming decreases rapidly with increase in the speed attained. The lower speeds, such as can be sustained for periods of the order of hours, have commonly been termed 'cruising speeds'. Some values for such basic speeds are already available in the literature. Magnan's (1930) figures for a variety of fish should probably be counted as of this nature. The average of his measurements for seventeen different common species, mostly marine, is $3{\cdot}2L$/sec., where $L$ is the body length. Fry & Hart's (1948) figures for goldfish (*Carassius auratus*) are based on periods of swimming of 20–25 min. and average at $6{\cdot}4L$/sec. Davidson (1949) gives $4{\cdot}0L$/sec. for salmon (*Salmo salar*) and Radcliffe (1950) $3{\cdot}4L$/sec. for goldfish tested over 25–30 min. Similar, but slightly lower, values can be calculated from information about the long-range migrations of fish.

More recently several workers have deliberately set out to relate speed to endurance. Paulik & DeLacy (1957) give values for sockeye salmon (*Oncorhynchus nerka*), silver salmon (*O. milktschitsch*) and steelhead (*Salmo gairdneri*) tested in a

rotating tank of water. With the speed of the tank gradually increasing, individual fish were observed until they began to lose laps. The speed preceding this was then recorded as one that could be maintained for long periods. The mean value for fifteen sockeye averaging 20 in. in length was 3·0*L*/sec.; for nine silver salmon averaging 22 in. in length 3·4*L*/sec. and for ten steelhead, averaging 25 in., 3·3*L*/sec. These may be taken as cruising speeds. In another series of experiments silver salmon and steelhead were tested to see how long they could swim before exhaustion in water velocities ranging from 4 to 10 ft./sec. These results, on fish about 24 in. long, give accurate values for sustained swimming lasting up to about 5 min. No observations were made over periods less than about 20 sec., but the results accord well with the figures reported in this paper for periods from 1 to 20 sec. and are discussed later.

In a second paper Paulik & DeLacy (1958) make it clear that the ability of sockeye salmon to swim persistently at various velocities is greatly reduced as a result of previous exertion. They demonstrate significant changes in the length of time that fish will swim at certain selected current velocities as a result of their migrating some 350 miles up the Columbia river and climbing to a height of 1800 ft. The maximum mean time swum at the lowest velocity (6·6 ft./sec.) in a straight flume was 196·4 sec. and this figure, for fish of mean length *c.* 20 in., represents a speed of 4·0*L*/sec.; their highest value, 9·4 ft./sec. for 64·9 sec., converts to 5·6*L*/sec. and both these values seem intermediate between long sustained cruising speeds and the bursts now being measured.

Brett, Hollands & Alderice (1958) have made a study of coho salmon (*Oncorhynchus kisutch*) and sockeye in a rotating circular chamber, relating cruising speed to temperature. Defining cruising speed as the maximum speed maintained for 1 hr. under strong stimulus, their values for 5·4 cm. long coho salmon at 20° C. is 30 cm./sec. (5·5*L*/sec.); for similar fish at about 0° C., 6 cm./sec. (1·1*L*/sec.); and for 6·9 cm. sockeye, 35 cm./sec. (5·1*L*/sec.) and 12 cm./sec. (1·7*L*/sec.) at the same two temperatures. They further show that these fish, if trained and exercised, will give significantly better performances.

Finally, Blaxter & Dickson (1959) give figures for the 'average maximum speeds' of a variety of marine fish tested in flowing and still water of various kinds. Their criterion for the inclusion of results was that the fish swam at least ten times their own length. No record of the duration of these tests is given, but Mr J. H. S. Blaxter has been so kind as to inform me that the speeds reported were usually sustained for 2–5 sec. Sea trout (*Salmo trutta*) of 12 in. in length are given as travelling at *c.* 10 ft./sec. (10·0*L*/sec.) and herring (*Clupea harengus*) 10 in. in length at *c.* 5 ft./sec. (6·0*L*/sec.). 20 in. cod (*Gadus callarias*) are slower at only 2·41*L*/sec. but this is probably a more sustained speed. Swimming various fish to exhaustion, the authors express further results in total body lengths travelled. The maximum of these is 1121 lengths for a batch of herring from 20 to 25 cm. in length. I am further informed that the average time for this performance was 13 min., giving a mean speed of 1·5*L*/sec. This is necessarily somewhat low because of turning time at the ends of the trough in which the tests were made.

Messrs R. W. McCauley and J. F. Skidmore of Ontario have also kindly provided me with hitherto unpublished data, on the cruising speed of the spawning phase of the sea lamprey (*Petromyzon marinus*). For 16 in. fish at 2° C. their figures give 0·57*L*/sec. and at 15° C., 0·89*L*/sec. Each of these values derives from the mean result for ten animals tested in Fry & Hart's (1948) original chambers.

Mr W. Muir of Garve, Ross-shire, has further given me figures obtained in a most ingenious manner by timing shoals of migrating salmon over a measured distance. This was done while watching them through field glasses from a height above the water during the direction of fishing operations. The mean value for fish of 24 in. mean length, swimming over a distance of 100 yards, is 2·0*L*/sec. It is clear from Mr Muir's report that this speed could be maintained for long periods of time. When startled the fish would spurt up to 4·2*L*/sec. for 30 ft. or so.

All the figures given above are of the same order of magnitude and it would seem substantiated that most of the fish so far considered can sustain speeds of the order of three to six times their body length per second for long periods. However, none of the techniques used to produce these results permits the accurate recording of sudden bursts. The fish wheel, allowing as it does for the almost instantaneous acceleration or deceleration of the water in which the fish is swimming, lends itself particularly to a study of the more violent periods of activity below about 30 sec. duration.

## EXPERIMENTAL METHOD

In order to facilitate the continuous recording of speeds over longer periods than hitherto the apparatus (Bainbridge & Brown, 1958) was modified by the addition of a permanent recording device and the filming of the fish itself was dispensed with. A standard Cossor double beam cathode-ray oscilloscope was linked to the electrically operated speed meter of the fish wheel, the voltage supply utilized being directly dependent upon the speed of rotation of the wheel. One spot of the oscilloscope tube was thus used as a direct indicator of the speed at which the fish was swimming and the other as a time marker. A continuously running paper-film camera attached to the oscilloscope then allowed a permanent record of speed against time to be made for any period of swimming. Such records are shown for a goldfish and a dace in Fig. 1.

In a typical experiment the wheel is filled with water at the recorded room temperature and a fish is introduced. After a period of 5–10 min. for settling down in the apparatus the fish is induced to swim and the wheel is rotated to keep him stationary at the observation point. Prolonged periods of steady swimming and shorter faster bursts can be variously induced by judicious use of a mild electric shock introduced into the wheel through a ring commutator and four diametrically placed brass plate electrodes on the floor of the Perspex tube. The shock is manually controlled through a pressure switch. Its intensity is regulated by means of a Variac; low voltages are generally used for large fish and higher voltages for smaller ones. Administration of the shock usually produces a burst of forward swimming of velocity very roughly related to the intensity of the shock. Such a burst is

followed by rotation of the wheel and is thence recorded on the oscilloscope. Occasionally a fish responds to the shock by stopping swimming or by turning round. Such specimens are not always consistent in their response and on other occasions may be more tractable. Low steady speeds are readily induced by rotating the wheel a little, so that the stationary fish is carried backwards over a striped background. The common optomotor response then results in a steady swimming to keep station and speed can be increased until the fish just starts to fall back. In contrast to the previously reported speed/frequency records, where the fish itself was photographed, the accuracy of the whole method on this occasion is entirely dependent upon keeping the fish stationary relative to the observer. At all times care has been exercised to ensure that this condition has been fulfilled.

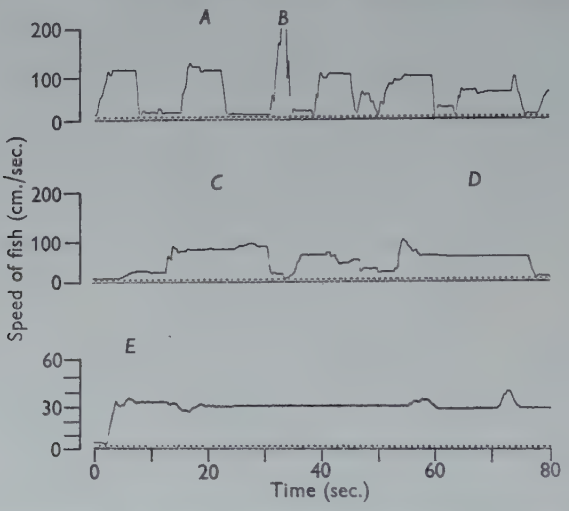


Fig. 1. Oscilloscope records of the speed of swimming of goldfish and dace showing *A*, a 5 sec. burst at 110 cm./sec., *B*, 1 sec. at 200 cm./sec., *C*, 15 sec. at 60 cm./sec. and *D*, 20 sec. at 50 cm./sec., all by a 13·5 cm. goldfish. *E* shows a 10·0 cm. dace swimming at about 30 cm./sec. for a longer period.

A particular fish is experimented with in this fashion over a period of several days, attempts being made to accumulate records of bursts of swimming of a variety of speeds and duration. The oscilloscope traces are then analysed to determine the maximum speeds that were sustained for a series of arbitrarily selected periods of 1, 2·5, 5, 10, 15 and 20 sec. These were chosen because of the relatively rapid initial fall off over the first few seconds and because by 20 sec. the low steady cruising speed seems generally to have been reached. In order to ensure that the absolute maximum speed has been recorded for any particular time period, it is necessary to repeat the observations again and again. Inspection of the records will eventually make it clear that a maximum has been reached, and such a speed can finally be attained on a good many occasions but never exceeded. Confidence in the experimental technique is further increased by considering the effect of size upon the results. From this it is clear that, while there is a general dependence upon size, individual specimens amongst a group of one size and a single species

have their own idiosyncrasies. Some will be more lethargic than others and will consistently give a poorer performance over a long series of observations to the extent of concealing somewhat the relationship with size (see below).

The oscilloscope traces are analysed using a transparent Perspex scale transferred from a calibration trace made with the wheel running at a series of convenient known steady peripheral velocities. This scale can be laid over the experimental trace and the parameters of any burst of swimming readily determined. No measure has been made of the rapidity of acceleration or deceleration. The speeds reported have been sustained at a constant level for the period of the observation. If the speed fluctuated during the period then the value accorded to that burst is the minimum reached during the period and not the average. This average would generally be only slightly higher than the figure recorded. Whenever possible absolutely steady speeds for the period involved have been selected. Typical readings, taken from the records illustrated, are given in the caption to Fig. 1.

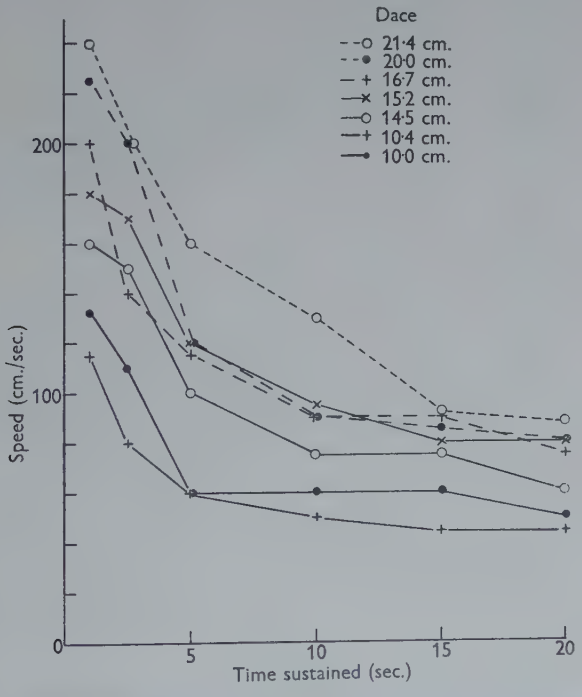


Fig. 2. Relationship between speed and the length of time it can be sustained for seven dace (*Leuciscus leuciscus*) ranging in size, as shown, from 21·4 cm. to 10·0 cm. in body length.

## EXPERIMENTAL RESULTS

### *The dace*, Leuciscus leuciscus

The first results to be examined relate to a series of seven dace ranging in length from 21·4 to 10·0 cm. Fig. 2 shows how their speed varies with the length of time for which the burst of swimming is sustained, up to a period of 20 sec.

Examination of the points for the 21·4 cm. specimen shows a rapid decline from a speed of 240 cm./sec., which is maintained for only 1 sec., down to 130 cm./sec. when the burst lasts for 10 sec. and 90 cm./sec. when it lasts for 20 sec. The flattening nature of the curve shows that a speed of 80 or 90 cm./sec. is probably one that can be maintained for some time. With the 10 cm. specimen the capacity to sustain speed declines with even greater rapidity, so that by 10 sec. of swimming the fish is already reduced to what is probably its cruising speed.

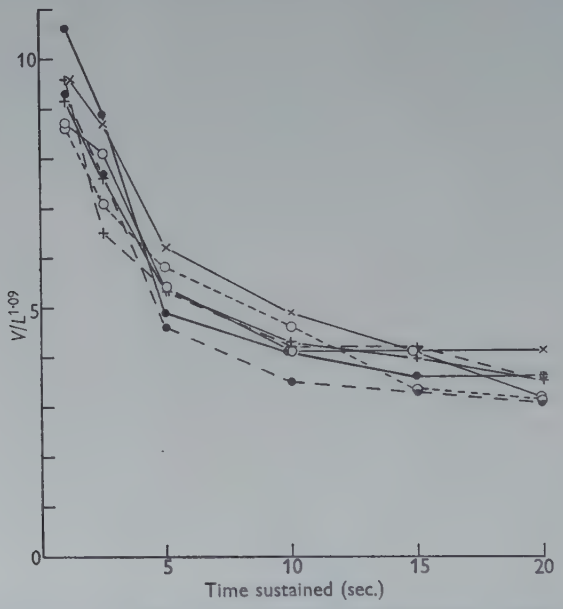


Fig. 3. Data shown in Fig. 2 with the speed in every case divided by the length of the specimen concerned raised to the power of 1·09. Symbols as in Fig. 2.

The figures for the specimens intermediate in size show the same type of relationship. Assuming that ability to maintain speed is some function of the length of these different sizes of fish, it is possible to express this relationship in the form $V/L^{\alpha} = F(T, \text{spp.})$, or $\log V = \alpha \log L + K_{T,\text{spp.}}$, where $V$ = velocity, $L$ = length, $F$ = a function peculiar to one species for a particular time interval. A plot of the logarithm of the velocity against the logarithm of the length, for the results for different sized fish at one particular time interval, should thus give a straight line whose slope is the value of $\alpha$. Repetition of this operation for the various time intervals gives six values of $\alpha$: 1·03, 1·15, 1·33, 1·11, 1·0 and 0·93 for the dace; the mean of these is 1·09.

Fig. 3 shows all the dace results of Fig. 2 treated on the assumption that the ability of different fish to maintain speed is dependent upon their length raised to the power of 1·09. The values of speed have in each case been divided by the size of the specimen, measured from the tip of the snout to the most posterior part of the tail, raised to the power of 1·09. These transformed values are again plotted

against time sustained and, as can be seen, give an acceptable correlation. The implications of this are considered later.

There is, however, a certain inconsistency in the correlation with size. The 10·0 cm. fish, for example, is substantially better in its performance than the 10·4 cm. specimen. This difference remains despite determined efforts to extract a better performance from the slower specimen. It may be accounted for by the possession of a more phlegmatic temperament or perhaps by the one fish being in poorer condition than the other. All the dace studied were born in the wild, but had been in captivity in roomy tanks for periods of months. There is no reason to suppose that they were in different condition as a result of immediate environmental influences.

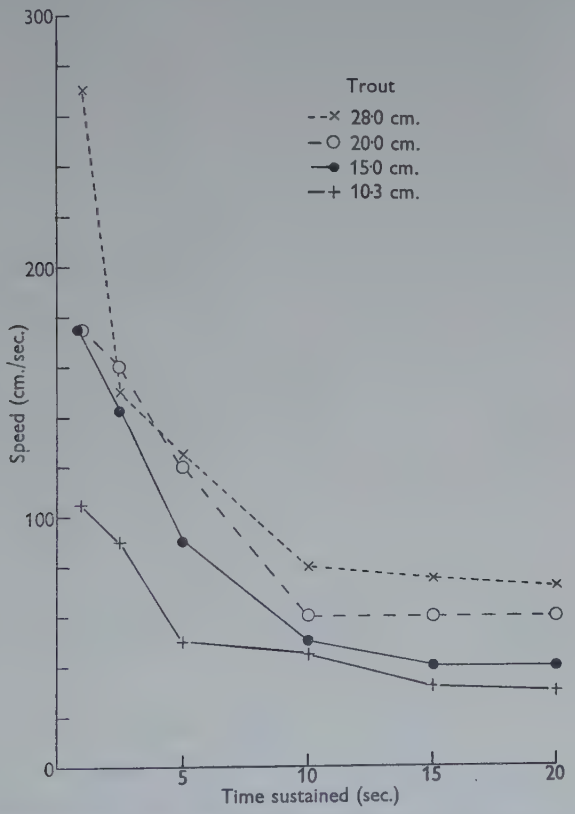


Fig. 4. Relationship between speed and the length of time it can be sustained for four trout (*Salmo irideus*) ranging in size, as shown, from 28·0 to 10·3 cm. in body length.

## *The trout*, Salmo irideus

Fig. 4 shows comparable results for four trout ranging in size from 28·0 to 10·3 cm. The same general form of the relationship between speed and the ability to sustain it is apparent. The spread of the observations is slightly greater with the greater size of the biggest fish. This specimen sustained 270 cm./sec. for 1 sec. and 72 cm./sec.

for 20 sec. The smallest fish, 10·3 cm. in length, sustained 105 cm./sec. for 1 sec. and only 30 cm./sec. for 20 sec.

Treatment of these results in the same manner as for the dace, to determine the nature of the relationship with length, gives the following values of $\alpha$ for the six time intervals: 0·71, 0·36, 0·70, 0·43, 0·65 and 0·65; the mean of these is 0·58. The trout records are thus shown in Fig. 5 with each value of speed divided by the length of the specimen raised to the power of 0·58. The correlation is particularly good for the lower values of speed. A direct comparison between these transformed results and those for the dace is not possible because two different powers of length have been used. Such a comparison is reserved until the goldfish figures have also been considered.

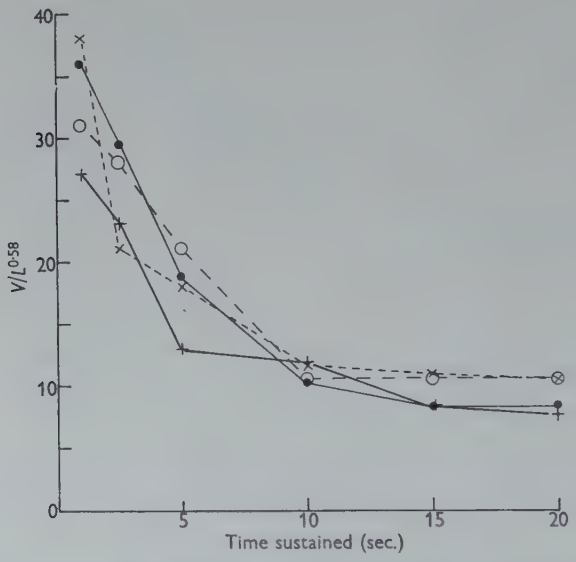


Fig. 5. Data shown in Fig. 4 with the speed in every case divided by the length of the specimen concerned raised to the power of 0·58. Symbols as in Fig. 4.

*The goldfish*, Carassius auratus

Fig. 6 shows values for a series of eight goldfish ranging in size from 21·3 to 6·7 cm. The general form of the relationship is as before, although the decline from the highest speeds appears to be slightly more gradual. Determination of the value of $\alpha$ as previously gives six figures: 1·06, 0·74, 0·76, 0·70, 0·48 and 0·50; the mean of these is 0·71. Fig. 7 shows the goldfish records of Fig. 6 with the values of speed divided by the length of the specimen raised to the power of 0·71. The correlation is again seen to be good.

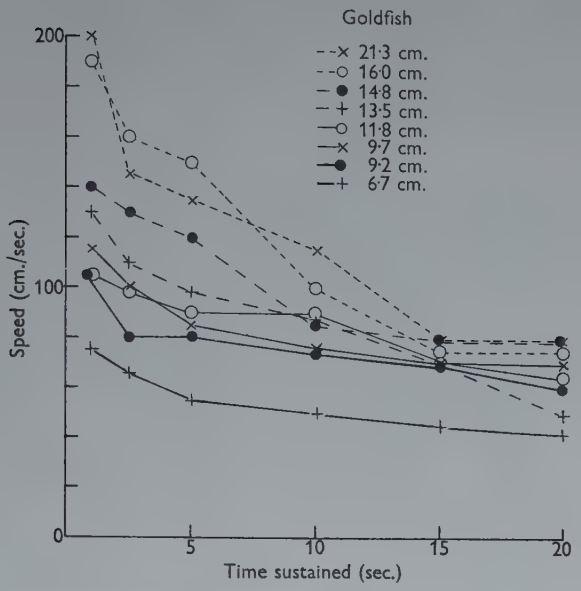


**Fig. 6. Relationship between speed and the length of time it can be sustained for eight goldfish (*Carassius auratus*) ranging in size, as shown, from 21·3 to 6·7 cm. in body length.**

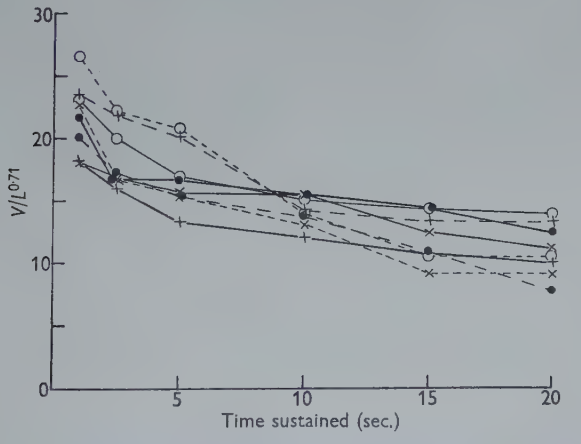


**Fig. 7. Data shown in Fig. 6 with the speed in every case divided by the length of the specimen concerned raised to the power of 0·71. Symbols as in Fig. 6.**

## DISCUSSION

Consideration of these experimental results reveals the complex interaction of various factors. In order to lend clarity to the argument the discussion has therefore been divided into several sections in which are considered separately: first, the effect of the size range in each species of fish tested; secondly, the effect of inter-specific differences; thirdly, the nature of the common relationship between time sustained and speed in all the species; fourthly, the correlation of the present results

with previous work, and lastly, some possible practical applications of the experimental observations. It becomes necessary from time to time to introduce further experimental data. This is done where the argument demands it rather than earlier, under the heading of experimental results.

### (1) *The effect of size*

Neglecting for the moment the exact form of the relationship between speed and duration for the different species of fish, we shall first consider the nature of its dependence upon size in specimens of different length. The three empirically determined values for the results obtained are: dace, $L^{1\cdot09}$, trout, $L^{0\cdot58}$ and goldfish, $L^{0\cdot71}$. Of these the second two are not found to be significantly different when a $t$ test is applied to the values and their standard errors; but the dace does differ significantly both from the goldfish and the trout. If the same relationship is assumed to hold for both the trout and the goldfish the mean value is $L^{0\cdot65}$.

In the present state of our knowledge it does not seem possible to provide a complete theoretical explanation of such a variable dependence upon length in different species. At least four factors would seem directly concerned: (*a*) the degree of non-isometric growth may vary between species, different sizes of fish being thus differently equipped with muscle; (*b*) the roughness of the surface may differ and have different proportional effects on laminar/turbulent flow at different sizes; (*c*) the fineness ratio and body form generally may vary with similar effects; and (*d*) the influence of Reynolds number must be considered. The first factor would influence the different amounts of power available to different sized fish, the remainder would influence the amount of drag encountered while moving through the water. Combined, these would necessitate different efforts at different speeds and hence result in variable staying power. They will each be considered in turn.

#### (*a*) *The effect of non-isometric growth*

Basically for a series of specimens of one species showing isometric growth, one can argue along various lines in order to determine how speed and the ability to sustain it may be related to length. In general, we may assume that the volume of the body and hence the proportionate amount of muscle increases as the cube of the length, while the surface area increases as the square of the length. From these two assumptions D'Arcy Thompson (1917) deduces that velocity must be proportional to $L^{0\cdot5}$. Making the further assumption that power is limited not by volume of the muscle, but by the surface area of the lungs or gills, he reaches an alternative conclusion that maximum velocity is constant and not related to length at all. Hill (1950) develops this same idea, adding the concept of heart capacity and blood flow through vessels whose cross-sectional area increases as the square of length, and also arrives at the conclusion that maximum speed is independent of length. While respiratory factors may well be of some limiting consequence, it is clear from the present results that neither of these two simple relationships holds for the fish so far examined.

(i) *Relationship of weight and length.* The first modification of the above simple theory concerns the assumption that the mass of muscle is proportional to $L^3$. Le Cren (1951) makes it clear that the cube law concerning the weight of fish of different lengths is rarely obeyed, the exponent $n$ in the formula $W = aL^n$ usually lies between 2·5 and 4·0 and can vary during the life of the fish. There is no reason to suppose that this deviation from 3 concerns only parts of the body other than muscle; although the relative weight of the gonads does change considerably in association with the breeding cycle. All the fish used in these experiments were therefore weighed and measured and $n$ was found to be 3·2 for the goldfish, 3·0 for the trout and about 2·8 for the dace. These values, unfortunately, in no way clarify the problem. The goldfish with the most favourable muscle/length ratio has the intermediate value for dependence of staying power upon length ($L^{0·71}$); the dace, with the least favourable muscle/length ratio has the highest value for staying power ($L^{1·09}$). In any case a disproportionate increase in bulk with increase in length means a corresponding increase in surface area and hence in the value for total drag. The opposing effects of these two tendencies might be to some extent mutually cancelling.

(ii) *Relative percentages of muscle.* A deviation from the principle of isometric growth that would not be self-corrective in this way would be a change, within a series of specimens of otherwise normal weight/length relationship, in the quantity of muscle relative to the remaining skeletal, digestive, nervous, etc., tissue of the fish. Information concerning such a change does not seem readily available in the literature, although Jacquot & Creac'h (1950) give references recording the *mean* proportion of muscle and its chemical constituents in many different edible fish. Reay, Cutting & Shewan (1943) give a figure for only one of the fish we are studying—trout—with 63% of muscle including skin. This accords well with the values reported below. Their figure of 33% for the perch is also interesting in comparison with our goldfish values.

All the propulsive trunk musculature was dissected from a series of dace, trout and goldfish. Some of the fish were freshly killed and lightly boiled to facilitate removal of the muscles, others had been fixed in formalin some time previously. All were weighed and measured both while fresh and after treatment. The different types of treatment had no determinable effect on the final results. The muscular and remaining components were weighed separately in a wet state after removing excess water by exposure in a standard manner to reduced pressure with a filter pump. It did not prove possible to separate the skin readily in all cases and its weight is therefore included with that of the muscle. From cases where the separation was made this would seem to introduce a consistent error of the order of 2%. From the figures the percentage wet weight of muscle, relative to the wet weight of the total fish, was calculated for each specimen. The results are summarized in Fig. 8 which demonstrates a general increasing trend in the proportion of muscle to total body weight. With increasing size, from the smallest specimens measured up to about 17·5 cm., there is an increase of about 10–15% in the relative amount of muscle in all species studied. Specimens within this size range form an

important proportion of the fish tested in the wheel. At a length of about 17·5 cm. the percentage of muscle appears to become roughly constant, although still showing some variability. The average proportions at this constant level are different for each of the three species and the significance of this is considered later.

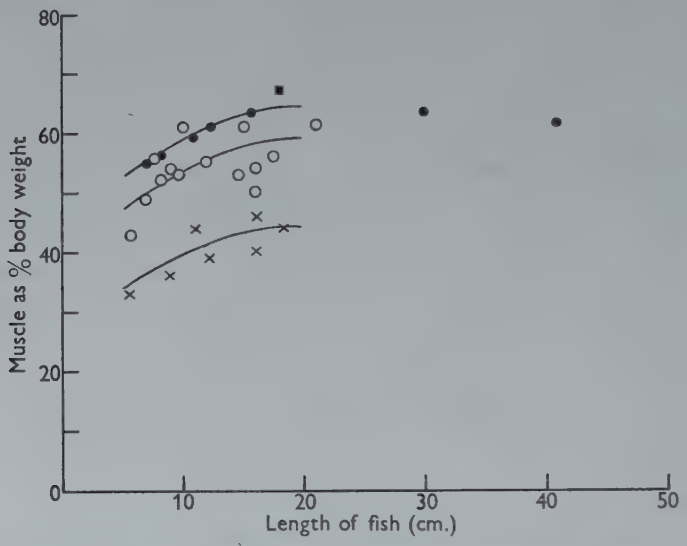


**Fig. 8.** Wet weight of propulsive trunk muscle as a percentage of total body weight plotted against length for various fish: dace (white circles), trout (black circles) and goldfish (crosses).

If these figures for change in muscle proportion with length are incorporated with those concerning the deviation of weight from the cube law (see above), the combined relationship between length and weight of muscle is then: dace $L^{3\cdot04}$, trout $L^{3\cdot06}$ and goldfish $L^{3\cdot40}$. There is thus in this respect little deviation from the cube law except in the goldfish, which changes advantageously with respect to power as size increases.

(*b*) *The roughness of the body surface*

There being nothing else immediately determinable in the propulsive mechanism of the fish that could account for the observed variations with length, we turn to consider the factors affecting the drag of the body. These fall under three headings, the first concerning the nature of the body surface itself. The layer presented immediately to the water would seem invariably to be one of mucus secreted by glands in the skin; beneath this is the epidermis, consisting of several layers of cells; and beneath this again, and largely determining the contours of the epidermis, is a layer of scales. The pattern of distribution of scales is more or less constant for a particular species. The full complement of scales appears early in life (in the speckled trout, *Salvelinus fontinalis*, scales have spread over practically the entire body by the time the fish is 6·0 cm. long—Elson (1939)) and thereafter the scales

increase in size by isometric growth in regular proportion to the growth of the whole body. The number of scales lying along the lateral line may be taken as some indication of the degree of roughness of the body. In the goldfish this is about 28, in the dace 52 and in the trout 126. The maximum lengths normally reported as being reached by these three fish are 40, 25 and 60 cm., respectively. This makes their maximum scale size about 1·5, 0·5 and 0·5 cm., respectively. The minimum scale size, when the pattern is first fully developed at say 6·0 cm., would be goldfish 0·2 cm., dace 0·1 cm., trout 0·05 cm., approximately.

The scales, overlapping as they do, will thus impart to the surface, though covered by epidermis and mucus, a pattern of roughness varying between these limits and changing in a uniform manner with the length of the specimen. The likelihood of this variation in roughness affecting the drag of the body in the way necessary to account for the observed variations of swimming ability with size is extremely small. Richardson (1936) has already shown that wooden models of fish respectively roughened, smoothed and varnished, and oiled were all of practically the same resistance as a dead fish. The variability in roughness of his models is greater than that within the size range of any one of the species we are considering and, if the roughness varies isometrically, its effects will in any case be covered by Reynolds number.

(*c*) *The effect of the fineness ratio*

Second in the factors affecting drag, the fineness ratio (F.R.) of the body may be considered. For a three-dimensional figure this is the ratio of the length of the body to the mean of its maximum height and breadth. With isometric growth F.R. might be expected to remain constant with increasing size, but with allometric growth it should either increase or decrease according to the nature of the departure from isometry. Two components are recognized in the drag of a three-dimensional body. The *frictional resistance*, which is usually expressed in terms of a coefficient based on the total wetted surface area, and the *form drag*, usually referred to the frontal area. These two factors contribute in varying measure to the total drag of the body in proportions dependent upon Reynolds number and the shape of the body. The total drag coefficient, based on frontal area, varies with a changing F.R. For airships where a premium is placed on volume in relation to drag, $C_f$ is at a minimum for an F.R. of 2·5 (Hoerner, 1958). It would seem likely that the case of fish is analogous, as an optimum volume for minimum drag will allow the maximum speed to be obtained. Any trend towards or away from an F.R. of 2·5 should therefor lead respectively to increasing or decreasing speed or stamina. Values for the F.R. for most of the fish used in these experiments, and some other specimens, are shown in Fig. 9. A decrease with increasing length is apparent in both the goldfish and trout, while in the dace there is a possibility of a slight increase although this is masked by the rather large variability. These changes (4·75–4·25 in the goldfish series and 6·40–5·70 in the trout) can be expected to reduce the total drag of these fish by about 4 and 5%, respectively. This is not of great significance. The marked difference in the absolute values of F.R. for the three species is, nevertheless, of

greater interest, the mean being 6·7 for the dace, 6·3 for the trout and 4·5 for the goldfish. Their significance is considered below.

*(d) The influence of Reynolds number*

The third factor which may influence the drag of the body is Reynolds number ($R$). In the simple expression Drag $\propto C_f L^2 V^2$, $C_f$ is a function of $R$. $R$ is expressed as $VL/\nu$ where $V$ is the velocity, $L$ the length of the specimen and $\nu$ is the kinematic viscosity of the water. The experimental values of $V$ make it clear that $R$ varies within each of our series of fish results. The limits of this variability are given in Table 1.

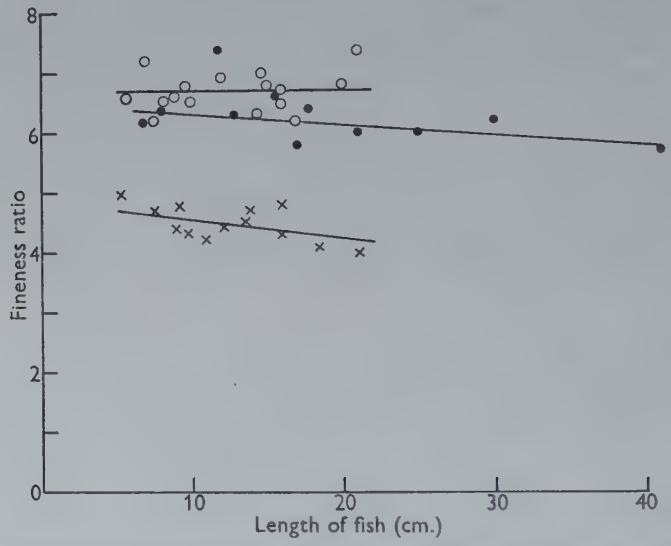


Fig. 9. Fineness ratios of various fish plotted against length for: dace (white circles), trout (black circles) and goldfish (crosses).

Table 1. *Limiting values of Reynolds number for the fish studied*

| | | Reynolds number | |
|---|---|---|---|
| | | Minimum | Maximum |
| Dace | Smallest | $4{\cdot}7 \times 10^4$ | $1{\cdot}4 \times 10^5$ |
| | Largest | $1{\cdot}8 \times 10^5$ | $5{\cdot}1 \times 10^5$ |
| Trout | Smallest | $3{\cdot}1 \times 10^4$ | $1{\cdot}1 \times 10^5$ |
| | Largest | $2{\cdot}1 \times 10^5$ | $7{\cdot}8 \times 10^5$ |
| Goldfish | Smallest | $2{\cdot}8 \times 10^4$ | $5{\cdot}0 \times 10^4$ |
| | Largest | $1{\cdot}7 \times 10^5$ | $4{\cdot}3 \times 10^5$ |

The coefficient of frictional drag relating to the wetted surface of a body moving in water varies according to the value of $R$, but there are unfortunately no measured values of the $C_f$ of bodies of revolution for the low Reynolds numbers pertaining to our fish. Unless there is some unexpected influence of the roughness of the surface it seems best to assume laminar flow over most of the body at these low Reynolds numbers. The theoretical laminar $C_f$ (based on wetted area) falls roughly

from 0·008 to 0·0015, while $R$ goes from $3 \times 10^4$ to $8 \times 10^5$. This disregards the effect of a changing F.R. With turbulent flow and a fixed F.R. of 5 the comparable change in $C_f$ would be from 0·015 to 0·0025.

These changes are substantial and have considerable effect when incorporated in the theoretical relationship between velocity and length. D'Arcy Thompson's arguments can be used to derive the expression $V \alpha L\{\frac{1}{2}(\beta-2)\}$, where $\beta$ is the disputed index in the formula which relates power available to length. Using modern hydrodynamical theory incorporating the effect of Reynolds number and assuming laminar flow over most of the body with $C_f \alpha R^{-\frac{1}{2}}$, the comparable expression is $V \alpha L\{\frac{1}{5}(2\beta-3)\}$. Incorrect assumptions concerning the effect of $R$ are undoubtedly a source of error in D'Arcy Thompson's deductions. The choice of assumptions concerning $\beta$ are (i) that it is simply dependent upon muscle volume (broadly $L^3$), or (ii) that it is entirely limited by the surface area of gills or such structures (broadly $L^2$). These two values for the index would now give $V \alpha L^{0 \cdot 26}$ and $L^{0 \cdot 2}$, respectively, instead of $L^{0 \cdot 5}$ and $V$ independent of $L$ as in D'Arcy Thompson's analysis. Substituting our three figures for mass of muscle (dace $L^{3 \cdot 04}$, trout $L^{3 \cdot 06}$, goldfish $L^{3 \cdot 40}$) would give velocity as $\alpha L^{0 \cdot 62}$, $L^{0 \cdot 62}$ and $L^{0 \cdot 76}$, respectively. Of these figures the trout and goldfish values tally well with our experimental findings but the dace is surprisingly low. Quantity of muscle is, however, probably not the limiting factor but without a careful study of relative changes in gill surface areas, circulatory systems, etc., it is not possible to say just how the power factor relates to length. Accepting the experimental results as reliable we can perhaps best at this stage only restrict our conclusions to a recognition of an empirical difference between the dace on the one hand (speed and ability $\alpha L^{1 \cdot 09}$) and trout and goldfish on the other ($\alpha L^{0 \cdot 65}$).

In the absence of so much basic information concerning limiting factors, arguments such as these must be considered as only exploratory. The basic assumption of a similar dependence upon length for all periods of swimming may even not be valid. It is possible that a burst of swimming of only 1 sec. duration might be dependent only upon the volume of muscle and hence make $V \alpha L^{0 \cdot 6}$, while more sustained swimming would begin to depend upon circulatory and respiratory transfer and hence perhaps finally make $V \alpha L^{0 \cdot 2}$. There is an indication of such a change in the goldfish results (p. 136). The series of values for $\alpha$ with increasing time interval (1·06, 0·74, 0·76, 0·70, 0·48 and 0·50) show the required decrement. The other two species do not show this and the effect has been neglected because of the complexity of treatment it would demand. It should, nevertheless, be borne in mind as a possibility.

The discrepancy between these present experimental values and the direct dependence of speed upon length reported earlier (Bainbridge, 1958*a*) need cause no concern. The formula previously given for calculating the maximum speed incorporated both the length of the fish and the maximum frequency of beating of the tail. The latter was shown to decline differently in different species with

increasing size of the specimen. The maximum efforts for varying durations which are being studied here would not therefore be expected to be directly related to length. They should differ from this in a manner dependent upon variation in the maximum frequency of tail beat with size.

### (2) *The effect of inter-specific differences*

Because of this variable dependence upon length it is not possible to make a direct comparison between the different species and thence assess their relative staying powers and the different onsets of fatigue in different species. One way in which an indirect comparison may be made, however, is by transforming the values for the mean curves in Figs. 3, 5 and 7 to values for a representative fish of arbitrarily selected length. This is done in Fig. 10 for theoretical fish of 15 cm. length, a size within the range of the experimental results for all species. The deductions which may be drawn from such a comparison are limited, because fish of such an arbitrarily selected length will not necessarily be at comparable stages in growth or development of sexual maturity.

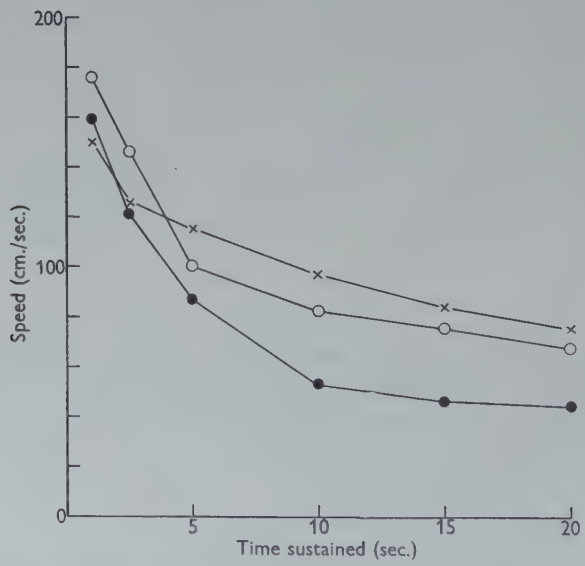


Fig. 10. Calculated relationship between speed and the length of time it can be sustained for three 15·0 cm. fish: dace (white circles), trout (black circles) and goldfish (crosses). Points derived from the means of the values in Figs. 3, 5 and 7.

Fig. 10 reveals certain differences between the three species which are perhaps somewhat unexpected. Starting with the shortest time interval of 1 sec. the dace is the best performer, the trout the next at about 15 cm./sec. or 13% slower, and the goldfish poorest at 10 cm./sec. lower still. With increase of the time interval to $2\frac{1}{2}$ and 5 sec. all three species show a marked decline in speed to about 60% of their maximum. The goldfish is by now, however, slightly better than the other two species. This ascendency is maintained with increasing time intervals, so that

for 20 sec. of swimming the goldfish maintains a speed almost double that of a trout of comparable size and almost 10 cm./sec., or 10%, greater than that of a similar dace. All species show a marked decline with time in the ability to maintain speed, the goldfish falling to 50% of its maximum value, the dace to 38% and the trout to 28%; the mean of these three figures is 40%.

Two pieces of information might correlate with this order of ability: the fineness ratios and the muscle percentages already calculated. In this context it is the differences between species that will be of consequence and not the changes with increasing length. With optimum laminar conditions, with $R$ at $4 \times 10^5$, the $C_f$ (based on frontal area) for the goldfish on the one hand at F.R. 4·5 is 0·048, while for the dace and trout on the other hand with F.R. *c.* 6·5 it is 0·065. These figures are greater than those already quoted for $C_f$ (wetted area) as they are based on different parameters of the body. This would lead one to expect a better performance (of the order of 10–20%) on the part of the goldfish, as is indeed found over the longer time intervals. Combined with this there must be some effect of the different percentages of muscle in the three species. The magnitude of this effect is difficult to assess because of the unknown influence of area-dependent factors such as the gills. If muscle volume alone were the determining factor it can be shown that this should depend upon (% muscle)$^{\frac{2}{3}}$. Treatment in this way of the mean muscle percentages for fish above 15 cm. (dace 56%, trout 63% and goldfish 45%) gives the following factors: dace 14·6, trout 15·8, goldfish 10·8. The goldfish with the most favourable $C_f$ has thus the most unfavourable muscle factor. When these two factors are combined the relative abilities should be: dace 21, trout 22 and goldfish 20. These are virtually identical and there would thus appear not to be any immediately determinable factors accounting for the observed variations in ability to sustain different speeds. The differences appear to be real and one may be forced to invoke some hitherto undetermined hydrodynamical or physiological factor to account for them . In this context Black (1955) has already made it clear that there are a number of interspecific physiological differences of as yet undetermined effect amongst fish. In particular his results show a large difference in the increase in blood lactic-acid content after 15 min. of forced exercise between carp, with low values (mean 65 mg. %) and Kamloops trout with high values (mean 91 mg.%). These he supposes must in turn be related to capacity to survive at different temperatures and possibly also to activity.

### (3) *Nature of the time/speed relationship*

Accepting these partly unaccountable variations related to size and species we may now consider more closely the nature of the relationship between speed and the time for which it is sustained. For this purpose size and species may be ignored and Fig. 11 gives the speed/time curve for a hypothetical fish of 15 cm. length. It is derived from Fig. 10 by taking the mean of the three values for each time interval. This mean curve shows that the fish have little power to sustain any of the higher speeds. The maximum speed, as recorded previously (Bainbridge, 1958*a*), is of the order of 10$L$/sec. This is kept up only for periods of the order of 1 sec. With 2½ sec.

of swimming it has already dropped to $7L$/sec., with 10 sec. to $5L$/sec. and with 20 sec. to $4L$/sec. The ability to sustain high speeds is thus much lower than that suspected previously. The form of the curve suggests that speeds of $12L$/sec. (180 cm./sec.) would be attainable by such a fish as this but these would be sustained for no more than 0·5 sec.

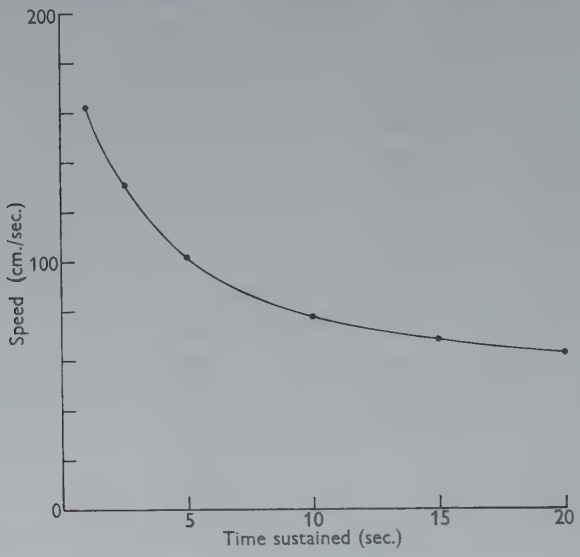


Fig. 11. Calculated relationship between speed and the length of time it can be sustained for a hypothetical 15·0 cm. fish. Points derived from the means of the values in Fig. 10.

It seems reasonable to suppose that there may be two factors contributing to determine the nature of this curve. With increasing time interval it clearly approaches an asymptote of between 3 and $4L$/sec. This must represent the 'cruising speed' of the fish and physiologically it must be more or less a steady state, determined itself by the rate at which the muscles can be supplied with the raw materials for contraction and relieved of their waste products. The rest of the curve, above a base line of $3–4L$/sec., represents potential available for a burst of swimming. This potential may perhaps be expressed in length-second units, of which about twenty appear to be available. These can be expended quickly or slowly according to the degree of motivation as, for example, an extra $4L$/sec. above the cruising speed if sustained for only 5 sec., an extra $2L$/sec. if sustained for 10 sec. or $1L$/sec. for 20 sec. The factors limiting the size of this potential may well concern a store of raw material available for use by the muscles (as glycogen within the cells themselves or oxygen bound in muscle haemoglobin or in close proximity to the cells), or it may perhaps depend upon the inhibitory nature of some waste product such as lactic acid. The rapid accumulation of this and its subsequent more leisurely removal would also account for the diminution in the 20 length-seconds potential as it is expended in shorter and shorter periods. (It is reduced to *c.* 15 length-seconds at $2\frac{1}{2}$ sec. and to 8 at 1 sec.) The uppermost limit of all to the speed of swimming is probably

determined mechanically by the strength of connective tissue junctions within the myotomes or perhaps by internal friction and viscosity within the fibres themselves.

Anaerobic conversion of glycogen to lactic acid is certainly one of the important mechanisms providing energy for muscular contraction. The appearance of such lactic acid in the blood of the fish appears to be somewhat delayed. Black (1956, 1957) gives information on the rate at which lactic acid appears in and disappears from the blood of various fish after violent exercise. He found no regularly determinable change in blood lactic-acid content up to 240 sec. after only 1 min. or so of violent activity associated with capture and the withdrawal of blood. Production must already have taken place in the muscles, however. During 15 min. of violent activity the mean rate of appearance in the blood was 10 mg.%/min. over the first 1–5 min. Normality might not be regained for as long as 12 hr. and this relates well to Paulik, DeLacy & Stacy's (1957) work on the effect of rest on the swimming performance of fatigued adult silver salmon. They found recovery after an exhaustive effort was only 31% complete after a rest of 1 hr., 43% after 2 hr., 67% after 3 hr. but not wholly complete until 18–24 hr. had elapsed.

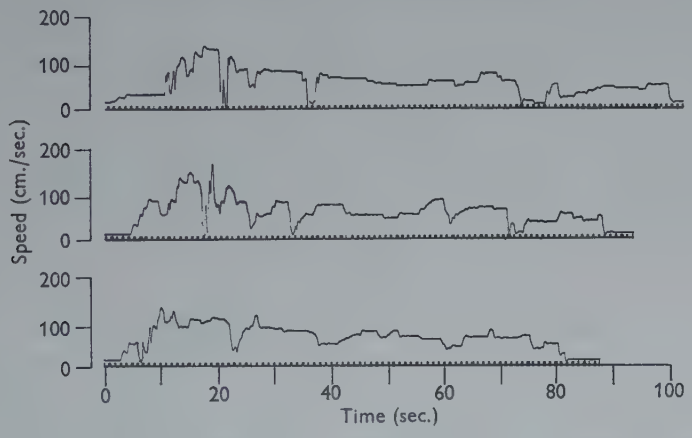


Fig. 12. Oscilloscope records of the speed of swimming of a 14·4 cm. goldfish showing three successive series of bursts of swimming lasting 80 or 90 sec. with an initial peak and a gradual decline indicative of fatigue.

The onset of fatigue must therefore be regulated by a critical balance between several variables including the ability of the contractile system to withstand high concentrations of lactic acid within itself or in its immediate vicinity, the speed with which this acid is removed from the muscles or is buffered, the speed with which it is transformed or removed from the body by the circulatory system and finally the intensity of the motivation influencing the fish at the time. The complexity of such a system of limiting factors could certainly account for both the form of the relationship shown in Fig. 11 and the interspecific differences already referred to. Another effect, not studied in detail, is illustrated by the record in Fig. 12. Here, over a period of about 1 min., a gradual decline can be seen in the speed attained during a series of successive bursts of swimming. This could presumably be determined

either by change in the rate of lactic acid removal as successive stages in the system become fully charged or perhaps by a nervous adaptation to the more or less uniform stimulus being received.

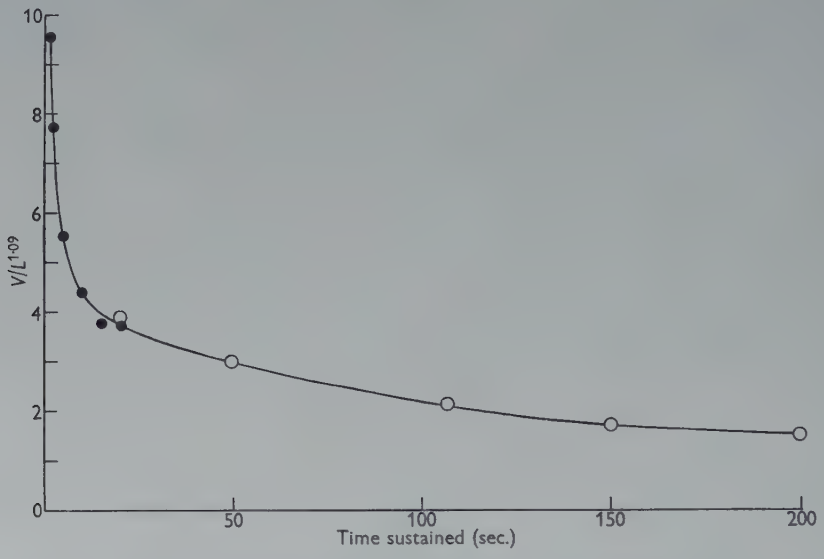


Fig. 13. Comparison between the present results for the dace (black circles) and results for the silver salmon, *Oncorhynchus milktschitsch* (white circles), from Paulik & DeLacy (1957). Both species with speed plotted as $V/L^{1\cdot09}$.

### (4) *Correlation with other results*

The figures described in this paper relate satisfactorily to those already published for longer time intervals. The tendency to approach a cruising speed of 3 to $4L$/sec. is evident in both the values in the introduction and in Fig. 12. Fry & Hart's (1948) high figure for the goldfish correlates well with the placing of the present goldfish results in Fig. 10. The mean cruising speed derived from Fry & Hart's figure and Radcliffe's (1950) figure is $4{\cdot}88L$/sec. That in our Fig. 10 for a 15 cm. fish represents $5{\cdot}0L$/sec.

Paulik & DeLacy (1957) show a gradual decline in the swimming ability of adult silver salmon over periods up to 200 sec. The shortest mean period of swimming they record is 24 sec. Their figures, the mean of nine fish, are reproduced in Fig. 13 on a scale of $V/L^{1\cdot09}$. Our dace figures are shown in the same notation. The complementary nature of these two sets of information is at once apparent. Although one might expect the correspondence of silver salmon to be greater with our trout this is not so, nor is it with the goldfish.

There is not of course such correspondence with observations on other animals. Hill (1950) gives the maximum recorded performances for man running races of different durations, man swimming and horses running. While the form of the relationship between speed and duration is precisely that found for fish in the

present work, the scales are of a different order, involving minutes in the mammal and only seconds in the fish. It is improbable that this striking difference derives from any essentially different type of muscular activity. A more likely explanation could lie in the relatively poor circulatory system of the fish. Ritchie (1928) makes it clear that the buffering capacity is very poor in fish muscle and rapid fatigue might be due to a change in pH because of an inability to remove lactic acid with sufficient speed. He further suggests that the glycogen reserve in fish muscle is extremely low and this would be a contributory factor. Black (1957) shows that after violent exercise the level of lactic acid in fish blood continues to increase for 2–3 hr., whereas in man this process continues for only 10 min. at the most. The low body temperature of fish may determine this slow rate of diffusion from the muscles. In mammals, correspondingly, the high body temperature will favour rapid transport of such diffusible substances.

Temperature of the ambient water is known to have a marked effect on the cruising ability of goldfish (Fry & Hart, 1948) and coho and sockeye salmon (Brett *et al.* 1958). No attempt has been made to study the influence of temperature in the present work. All the observations were made at room temperature and in every case this was recorded. The fish were held in tanks at the same room temperature and were therefore always acclimatized to the temperature of the water in which they were studied. For the purposes of comparison all the records may be taken as relating to 15° C., but on occasion observations were made as much as 3° C. on either side of this figure. Because of the limited number of these no consistent influence of temperature could be detected in the speeds recorded but this factor may account for some of the variability that was encountered.

### (5) *Practical applications*

Besides raising these various physiological problems the figures reported here may be of some practical significance. Two such possible applications are now considered. For this purpose it would seem best to neglect possible variation between species and refer to a hypothetical average fish derived from the mean of all the results so far obtained. The swimming abilities of the members of a series of such animals of varying sizes should be related to each other according to their length raised to the power of 0·8; this being the mean of the three indices already calculated. Using this power and the values in Fig. 11 it is possible to construct a graph relating the speed of such hypothetical fish to the maximum distance they could swim. Such a relationship is shown in Fig. 14 for four representative lengths of fish (15, 30, 45 and 60 cm.). 60 cm. (about 24 in.) has been chosen as the upper reliable limit for extrapolation of the results, which derive from fish whose maximum length is 30 cm.; and 24 in. is also a reasonable size for a spawning salmon.

If one of these animals, e.g. the 60 cm. specimen, is swimming head into a current of water the distance he can travel forward relative to the ground is given by the simple relationship $D = T(V_f - V_w)$, where $T$ is the duration of the burst of swimming, $V_f$ the velocity of the fish and $V_w$ that of the water. If this formula is applied to the data in Fig. 14 it is possible to derive the relationship shown in

Fig. 15. This gives the headway that a 60 cm. fish can make against various speeds of flowing water according to the speed at which he swims. It is at once apparent that for each water speed there is an optimum speed of swimming which will carry the fish a maximum distance. In water at 200 cm./sec., for example, for a 60 cm. fish, this is 325 cm./sec. At this speed he will make a headway of 545 cm. If he swims faster than this he fatigues before having travelled so far; if he swims slower he is

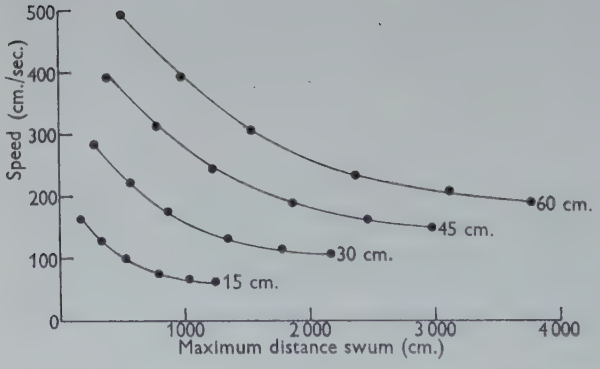


Fig. 14

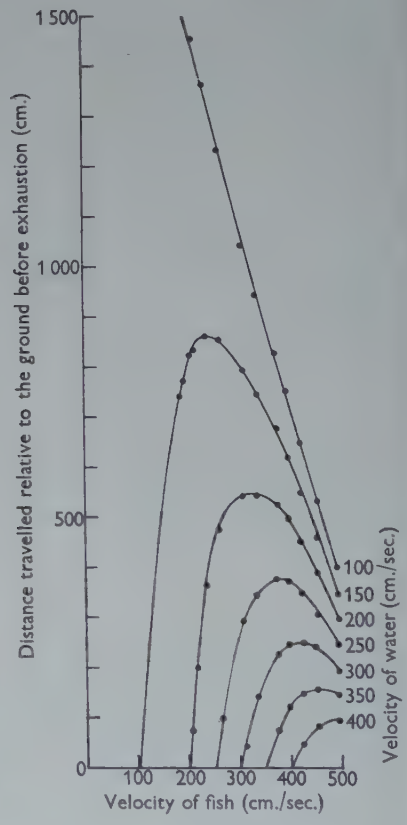


Fig. 15

Fig. 14. Relationship between speed and the maximum distance swum before exhaustion for four hypothetical fish of 15, 30, 45 and 60 cm. in length.

Fig. 15. Headway, relative to the ground, made by a hypothetical 60 cm. fish swimming at various speeds in currents of various velocities. The separate curves relate to the different current velocities; the black circles indicate distance travelled for particular speeds of swimming. For further explanation see text.

carried back too far to make as much headway. A more convenient way of presenting this information is by plotting the maximum distance that can be travelled against water velocity. This is done in Fig. 16 for the four sizes of fish; the data being derived in each case as in Fig. 15. This diagram, with scales in both cm., ft. and miles per hour, provides at once, for each of the sizes of fish listed, the absolute

maximum distance that such a specimen might be expected to traverse through water flowing at a particular speed. For example, in a current of about 3 m.p.h. (say 150 cm./sec.), 6 in. fish will have no chance of even stemming such water, 12 in. fish could traverse a maximum length of 6 ft. of it, 1 ft. 6 in. fish could traverse about 14 ft. and 24 in. fish about 26 ft. After such lengths of swimming a period of rest would be required before another effort. A somewhat reduced effort could be indulged in within seconds, an equally strenuous one possibly not for much longer.

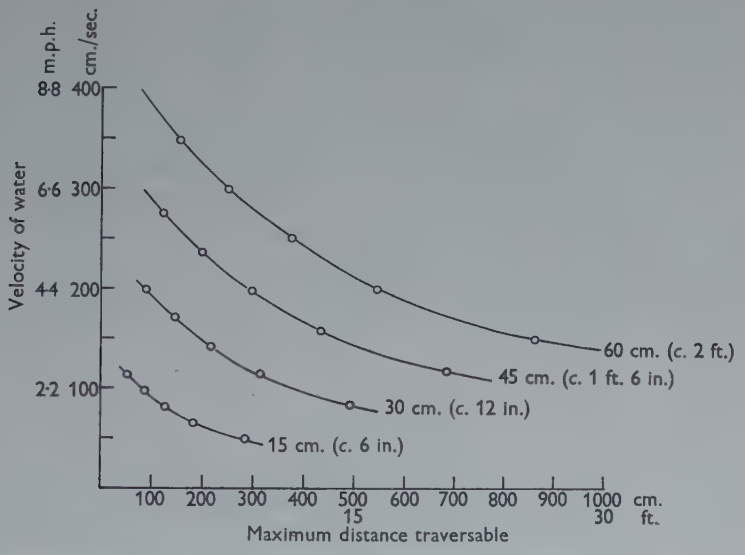


Fig. 16. Maximum distance traversable relative to the ground by four hypothetical fish swimming in water of various velocities. Data derived from Figs. 14 and 15.

The above argument assumes movement through water of constant velocity. Sir James Gray has shown (unpublished) that a fish entering water of increasing velocity could make use of the speed developed in a lower velocity region by travelling on his momentum quite a long way into the water of increasing velocity. Such an effect would tend to increase the distance that could be traversed. It is also important to stress that Fig. 16 represents an optimum effort on the part of the fish. If he swims either too fast or too slow he will not accomplish so much. Fig. 15 indicates the latitude he has, without seriously reducing his distance. In a 200 cm. current the 60 cm. fish will still cover over 500 cm. distance for speeds of swimming between 275 and 400 cm./sec. It is interesting to contemplate whether the fish has a means of judging his optimum by reference to, say, the bottom. It is certainly important that under conditions requiring a maximum accomplishment he should not be scared into trying to swim his fastest.

The application of such information as this to the passage of fish through ladders is at once evident. Maximum efficiency of a ladder is often related to the velocity of the outflow, as the faster this is the more fish it attracts. Adjustment to

an appropriate velocity could ensure entry of the maximum number of fish and also exclusion, if desirable, of those of smaller size. A second application might concern the dimensions of the openings of trawls and other nets. The modern otter trawl has a headline of the order of 100 ft. in width, which, during working, may be 10 or 15 ft. above the sea bed. It is towed along at a speed of 3 to 4 knots. Fig. 16 suggests that most fish below 12 in. would be likely to be caught by such gear. A fish of 24 in. would manage a dart of about 20 ft. at this speed. If he is within that distance of the edge of the net and moves laterally he will escape. Within the central 60 ft. of the line of towing he cannot escape except by swimming upwards, where he is easily capable of getting over the top of the net. This, however, generally overhangs the foot-rope by perhaps 10 ft.; its tendency will therefore be to make him swim downwards and along the sea bed, to be caught eventually after tiring. Laterally the Vigneron Dahl gear will increase the effective spread of the net and, preceding it somewhat will tend to scare laterally placed fish inwards where, after one of their sudden darts they will be more readily caught. Even much bigger fish will by this means be brought into the catching power of the net. It seems unlikely that the trawl, developed by long years of experience, can be improved upon by information of this kind but the results do perhaps serve to emphasize how deadly a device it already is and also show, partly, why this is so.

## SUMMARY

1. Measurements of the maximum speed sustained during bursts of swimming of up to 20 sec. duration by dace, trout and goldfish of various lengths are reported.

2. The ability to sustain periods of swimming appears to be related differently to length in different species. In the dace it is proportional to $L^{1 \cdot 09}$, in the trout and goldfish to $L^{0 \cdot 65}$.

3. The dependence of this relationship upon various factors is considered. It is concluded that allometric increase of muscle and the influence of an increasing Reynolds number, possibly combined with other, physiological, factors could adequately account for the variation observed.

4. The precise form of the speed/duration relationship differs in different species. These variations are also probably accountable for by a combination of different muscle percentages and fineness ratios together with other, physiological, factors.

5. The ability to maintain speed diminishes rapidly with increasing time interval in all the species measured. The maintained speed falls from about $10L$/sec., maintainable for only 1 sec. of swimming, down to $5L$/sec. for 10 sec. and further to a cruising speed of about $4L$/sec. by 20 sec. The nature of this time/speed relationship is considered to be determined by the ability of the muscle to utilize stores of raw material and by the rates at which these can be supplied to, and waste products removed from, the muscle.

6. A diagram showing the relationship between velocity of water flow and the maximum distance which various hypothetical fish could traverse in such currents is constructed; and possible applications of this information are briefly considered.

I am indebted to many kind friends for their encouraging interest and help; particularly to Sir James Gray who made the work possible, to Dr R. H. J. Brown for his technical help and advice and to Dr K. E. Machin for his continued patient assistance with the mathematics.

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# THE EFFECT OF PERTURBATIONS IN THE ENVIRONMENTAL CYCLE OF THE DIURNAL RHYTHM OF ACTIVITY OF *PERIPLANETA AMERICANA* L.

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## INTRODUCTION

It has been clearly established that the phases of the diurnal rhythms of most animals bear a specific relationship to the time of onset of darkness, or light, when these environmental factors are alternated within 24 hr. cycles. The completion of the light:darkness cycle is not, however, always necessary for phase determination. One short period of light will serve to establish a rhythm in some arrhythmic animals (Brett, 1955; Harker, 1958); the environmental change in this case contains no information concerning period, and the establishment of a 24 hr. response is strong evidence for an endogenous control of periodicity.

If a clear rhythm is already being shown by an animal the effect of a single alteration in the time of exposure to light or darkness might be expected to give information about the properties of the controlling mechanism of rhythmicity. Pittendrigh & Bruce (1957) have discussed the significance of the effect of single light perturbations on the resetting of phase in the eclosion rhythm of *Drosophila*, and have postulated control by an innate mechanism consisting of two coupled oscillators (Pittendrigh, Bruce & Kaus, 1958). The response of an oscillator to a non-periodic disturbance may give rise to transients, and it is this term which has been applied to the non-periodic peaks of eclosion which follow a single perturbation of the environment prior to the reappearance of a steady rhythm.

Some difficulties arise in the interpretation of Pittendrigh's results because the process measured is one which takes place only once in the life-cycle of each animal. In contrast to these results transients do not always appear in the rhythm of a continuous process after a non-periodic disturbance in the environmental cycle; phase shift may be immediate as is found in the rhythm of luminescence of *Gonyaulax*, a unicellular organism (Hastings & Sweeney, 1958).

A continuous-process rhythm in a more specialized animal than *Gonyaulax* might be supposed to involve a number of rhythms or cycles, some or all of which may be affected to a different degree by the environmental perturbation: the total effect, as measured by the reaction of the organism as a whole, might be that of a transient response. In this paper the effect of environmental perturbations on the locomotor activity rhythm of *Periplaneta americana* is described. This animal has been chosen because it is known that its activity rhythm is dependent on the rhythmic secretion of a hormone from the neurosecretory cells of the suboesophageal ganglion. The

phases of the secretory rhythm of these cells can be measured by implanting the ganglion into an arrhythmic animal which then becomes active at the time of secretion (Harker, 1956). By using this technique the effect of an environmental perturbation on two rhythms, the neurosecretory rhythm, and the locomotor activity rhythm, can be measured.

## EXPERIMENTS AND RESULTS

The locomotor activity of *Periplaneta* over 24 hr., when light and darkness are alternated in 12 hr. cycles, follows a fairly constant form in which six stages can be recognized (Fig. 1). Stage A. Some time before the onset of darkness the level of activity may rise. Stage B. After the onset of darkness the increase in activity is sudden and marked. Stage C. A period of 2–3 hr. in which activity is high. Stage D. The level of activity decreases over a period of about 2 hr. Stage E. The activity remains at a fairly low level. Stage F. About 5 hr. after the onset of light activity is at a minimum, or may cease completely, for an hour or two: this stage appears to be directly related to the change from darkness to light, for it does not appear in the rhythm of an animal kept in continuous light.

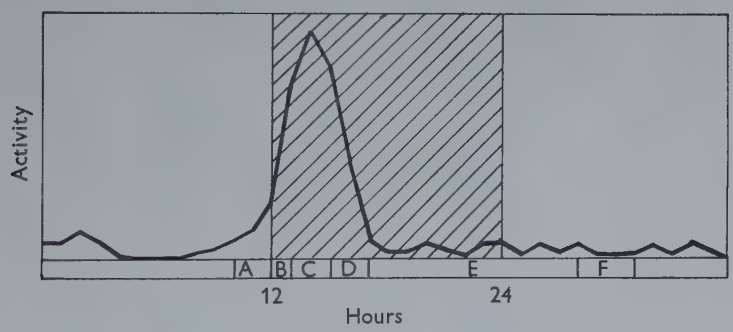


Fig. 1. The six stages of the locomotor activity rhythm of *Periplaneta americana* in alternating light and darkness. Hatched area represents dark period.

In each of the following experiments the phases of the activity rhythms of the cockroaches in each group were set by keeping the animals in 12 hr. light : 12 hr. darkness for at least 3 weeks. Hereafter these conditions will be termed 'normal', and a rhythm following the stages described above a 'normal' rhythm. The activity of two animals at a time was recorded in each of the new conditions, and each experiment was repeated five times. As a control the activity of one group of animals was recorded in the normal conditions at the same time as each of the experiments. Activity was measured using a photo-transistor recorder working on very dim red light (Brown, 1959), except in the case of two animals in each experiment whose activity was recorded by a direct method described in a previous paper (Harker, 1956). The two methods gave similar results in all experiments.

Group A. From previous experiments (Harker, 1958) it is known that the secretory activity of the suboesophageal ganglion starts at the beginning of the active

phase of the locomotory rhythm, and this in turn corresponds with the time of onset of darkness. In order to measure the effect of a change from light to darkness on the secretory cycle during the various stages of the activity rhythm groups of cockroaches were placed in darkness every 2 hr. over a 24 hr. period. Thirty minutes after the onset of darkness the suboesophageal ganglia were dissected out and implanted into arrhythmic headless animals, whose subsequent activity was recorded.

The times at which the arrhythmic animals became active, a measure of the time of neurosecretion, are shown in Fig. 2.

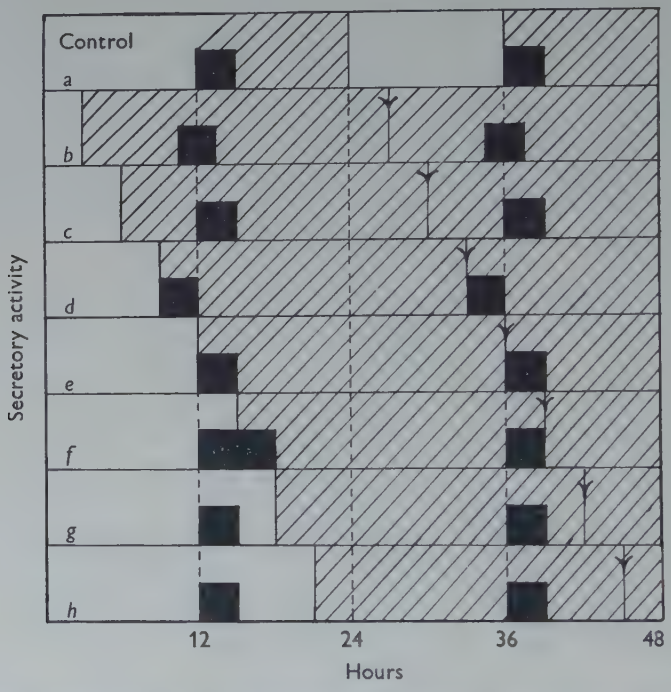


Fig. 2. The time of secretion from the suboesophageal ganglion neurosecretory cells after onset of darkness at times out of phase with the activity rhythm. The arrowed line represents the time at which the dark period began on the previous day.

From the results (also confirmed by those in the following section), it appears that there is a stage in the neurosecretory cycle when secretion will take place whatever the external conditions, a stage when secretion will take place if there is a change from light to darkness ('possible' secretion), and a stage when no secretion will take place even after such a change ('impossible' secretion). The stages of the cycle are represented diagrammatically in Fig. 3.

If a change from light to darkness occurs at a time when secretion is already taking place the phases of the cycle appear not to be reset in any way: secretion next occurs at the 'normal' time. If the onset of darkness occurs at a time when secretion would not normally be taking place, but when the cells are able to respond to the

stimulus, then the cycle is reset, so that secretion next occurs 24 hr. after the stimulus. If secretion cannot take place at the time of the stimulus then the neurosecretory cycle appears to be unaffected, and secretion continues to take place at the 'normal' time.

Group B. Further experiments were carried out under a variety of conditions as detailed below. The results are shown in Figs. 4–7, in which the lines *b–e* refer to the conditions so labelled hereunder. Line *a* in each figure is the result of a control experiment in which the 'normal' conditions, those in which all animals had previously been maintained, were continued.

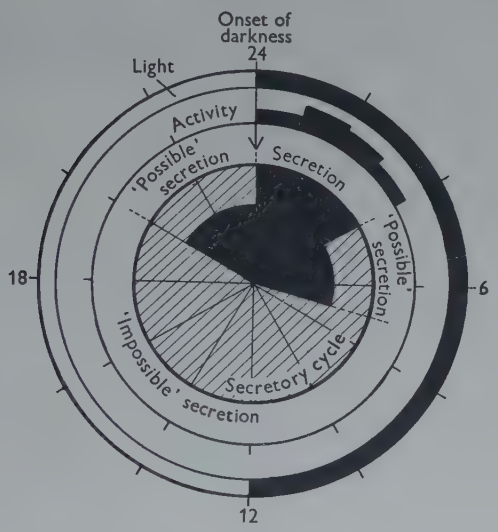


Fig. 3. Diagrammatic representation of the neurosecretory cycle in relation to time of darkness and locomotor activity.

(1) Onset of darkness 2 hr. earlier than normal (Fig. 4).

(*a*) Control.

(*b*) Normal 12 hr. darkness : 12 hr. light cycle set forward by 2 hr.

(*c*) Onset of darkness 2 hr. earlier than normal followed by continuous darkness.

(*d*) Onset of darkness 2 hr. earlier than normal, initiating a cycle of 4 hr. darkness: 20 hr. light.

(*e*) Onset of darkness 2 hr. earlier than normal followed by continuous light.

As an additional experiment, under some conditions, the suboesophageal ganglion was removed 2–4 hr. after the onset of darkness and implanted into an arrhythmic animal whose subsequent activity was recorded in continuous light (Figs. 4*c*, 5*b*, 6*c*, 7*b*).

(2) Onset of darkness 7 hr. earlier than normal (Fig. 5). *a*, *b*, *c*, *d*, *e* as under 1.

(3) Onset of darkness 4 hr. later than normal (Fig. 6). *a*, *b*, *c*, *d*, *e*, as under 1.

(4) Onset of darkness 8 hr. later than normal (Fig. 7). *a*, *b*, *c*, *d*, *e*, as under 1.

The following points arising from the results seem worthy of special mention.

(i) When the animals are kept in continuous darkness after the environmental perturbation the beginning of activity on successive days is 24 hr. later than the beginning of the previous active period, whether the previous active period resulted

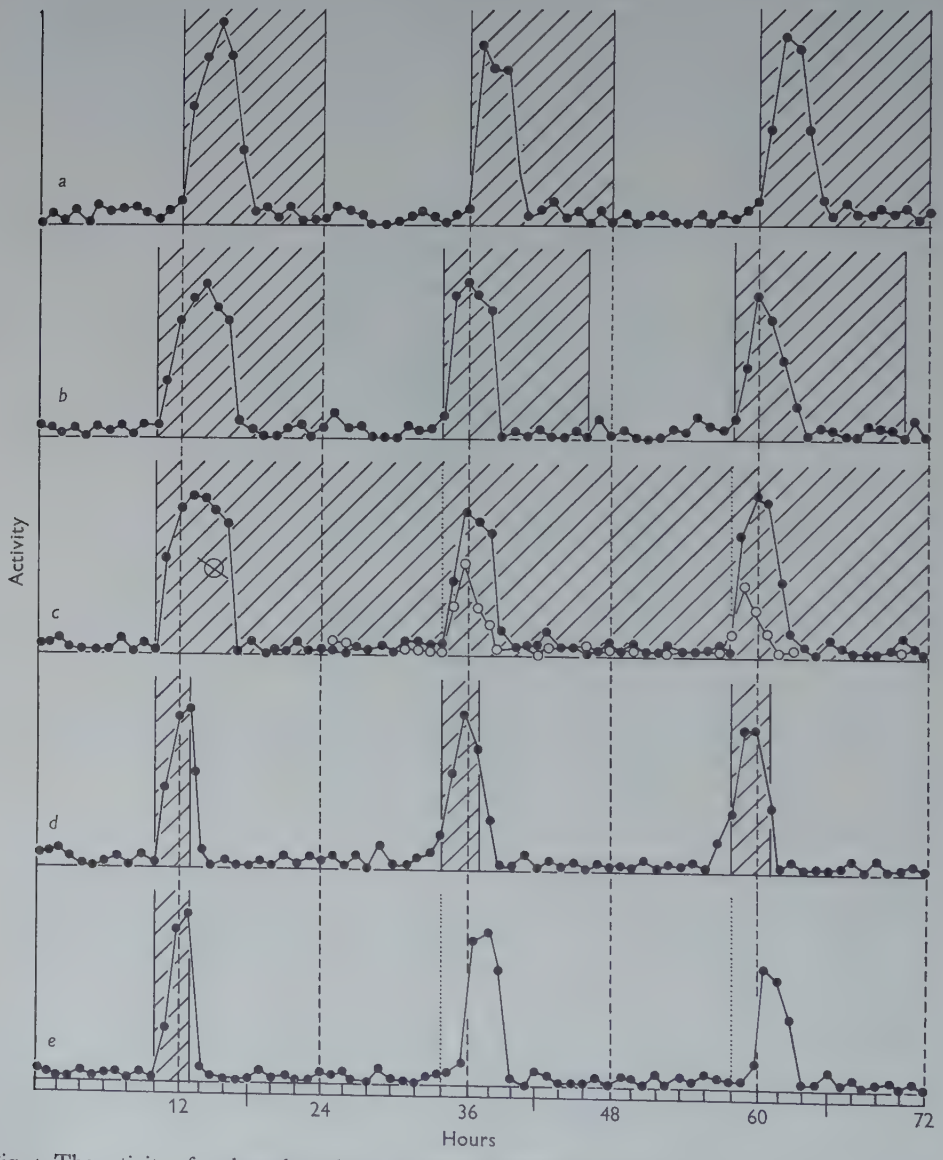


Fig. 4. The activity of cockroaches when: (*a*) onset of darkness occurs at the 'normal' time—control experiment; (*b*) darkness occurs 2 hr. earlier than previously experienced, new light: dark phases continued; (*c*) new time of darkness followed by continuous darkness; (*d*) 4 hr. dark period beginning at new time; (*e*) 4 hr. dark period at new time followed by continuous light. ⊖, Time at which ganglion removed and implanted into arrhythmic animal. ○–○, The activity of the implanted animal.

from the new or the old environmental condition. This is noticeable, for example, in the experiment in which the onset of darkness came 4 hr. later than normal (Fig. 6*c*); activity continued after this stimulus for a longer period than normal, but the timing of the subsequent peaks is related to the beginning of the normal peak, and not to that of the second peak produced by the new stimulus.

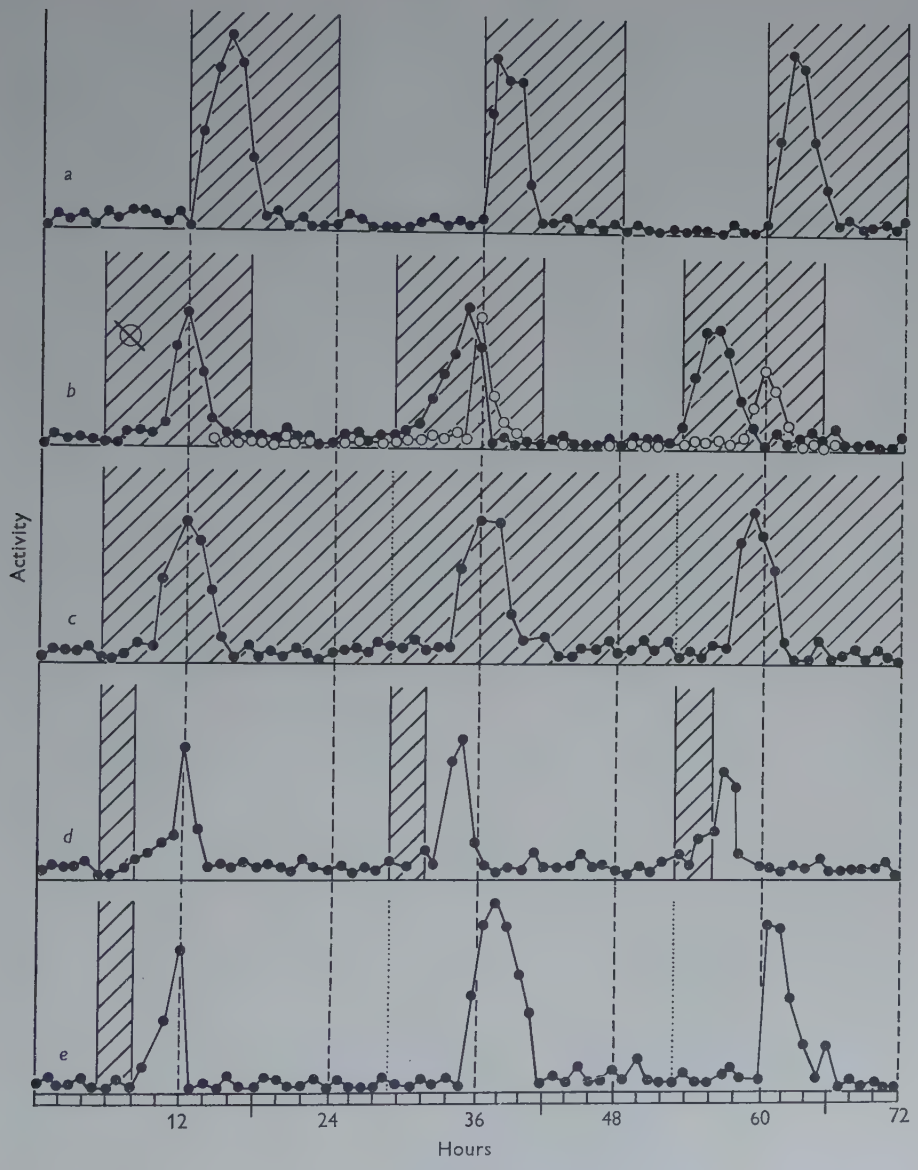


Fig. 5. The activity of cockroaches when: (*a*) onset of darkness occurs at the 'normal' time—control experiment; (*b*) darkness occurs 7 hr. earlier than normal, new light:dark phases continued; (*c*) new time of darkness followed by continuous darkness; (*d*) 4 hr. dark period beginning at new time; (*e*) 4 hr. dark period at new time followed by continuous light.

This result is in keeping with the finding that the secretory cycle is not reset by a stimulus occurring during the secretory phase (Fig. 2*f*).

(ii) When the onset of darkness came 8 hr. later than usual (Fig. 7*b*), or 7 hr. earlier than normal (Fig. 5*b*), the animal did not respond immediately by becoming active but the subsequent activity peak occurred 4–6 hr. earlier than normal.

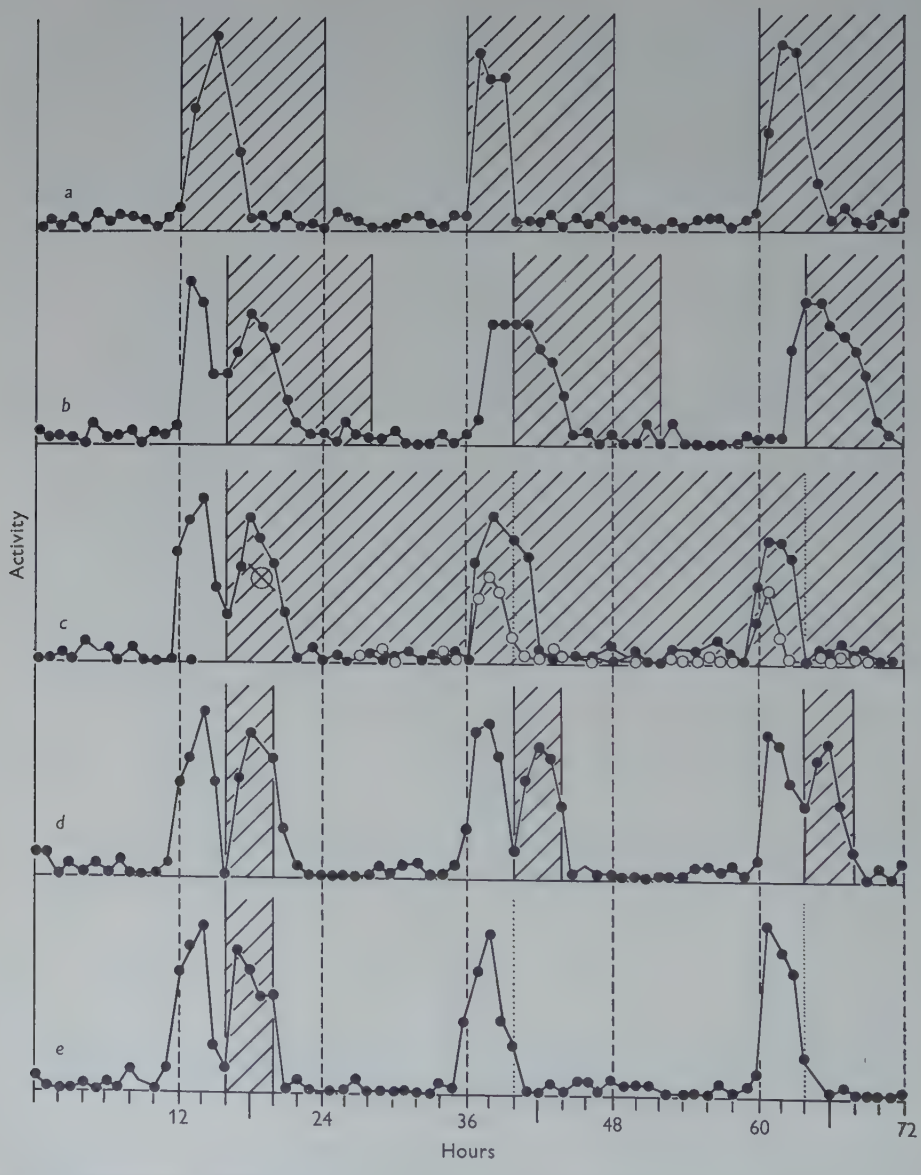


Fig. 6. The activity of cockroaches when: (*a*) onset of darkness occurs at the 'normal' time—control experiment; (*b*) darkness occurs 4 hr. later than normal, new light:dark phases continued; (*c*) new time of darkness followed by continuous darkness; (*d*) 4 hr. period beginning at new time; (*e*) 4 hr. dark period at new time followed by continuous light.

In the experiment of Fig. 7*b* the animals implanted with ganglia removed 4 hr. after the onset of darkness also showed an activity peak 4–6 hr. earlier than normal. This shows that although neurosecretion is not immediately evoked (the onset of darkness falling within the stage of 'impossible' secretion) there is yet some effect which brings forward the next episode of neurosecretion by 4–6 hr. In the com-

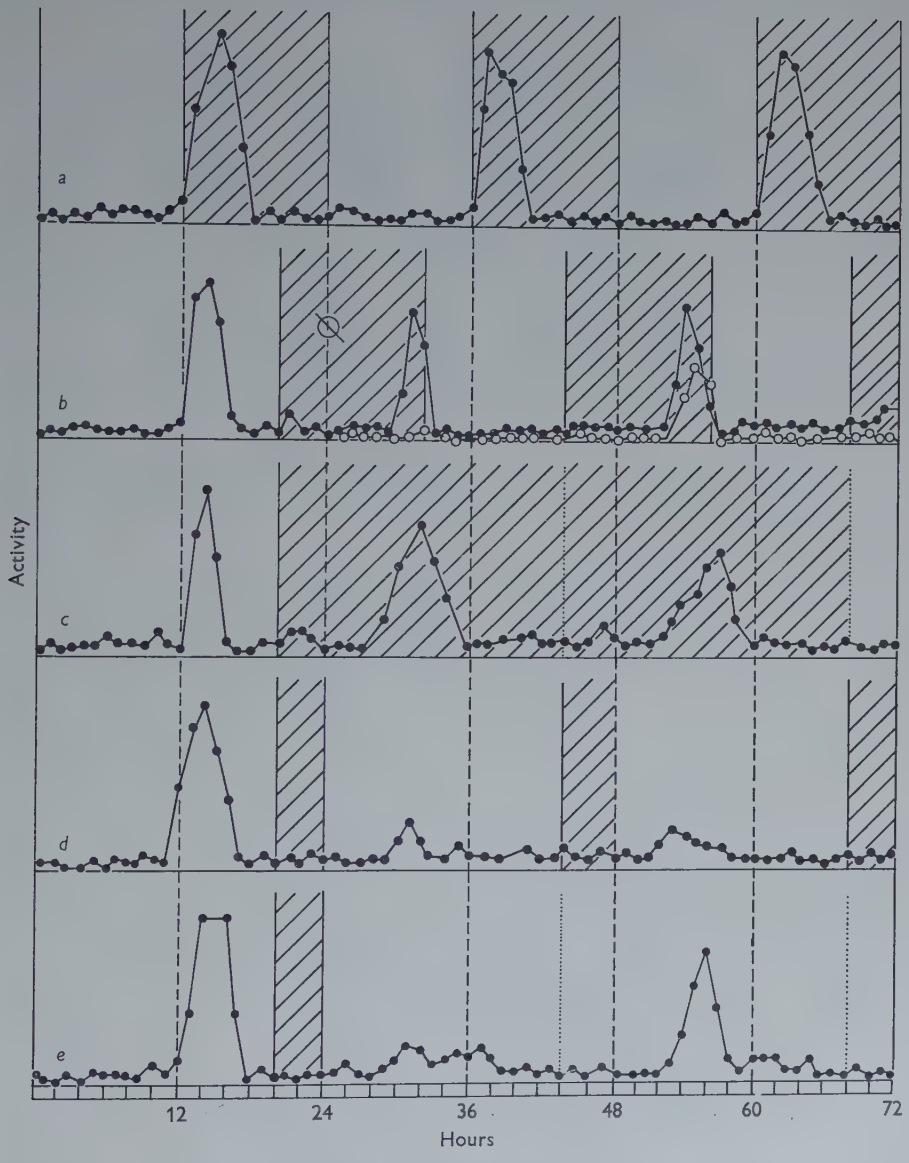


Fig. 7. The activity of cockroaches when: (*a*) onset of darkness occurs at 'normal' time—control experiment; (*b*) darkness occurs 8 hr. later than normal, new light:dark phases continued; (*c*) new time of darkness followed by continuous darkness; (*d*) 4 hr. period beginning at new time; (*e*) 4 hr. dark period at new time followed by continuous light.

parable experiment of Fig. 2*g* in which the ganglia were removed 30 min. after the onset of darkness, the next episode of neurosecretion was not brought forward. Nor was it brought forward in the experiment of Fig. 5*b*, in which the ganglia were removed 2 hr. after the onset of darkness. It appears that the effect of the onset of darkness in bringing forward the next episode of neurosecretion is a long-term one, in that it requires that the ganglia remain *in situ* for some hours.

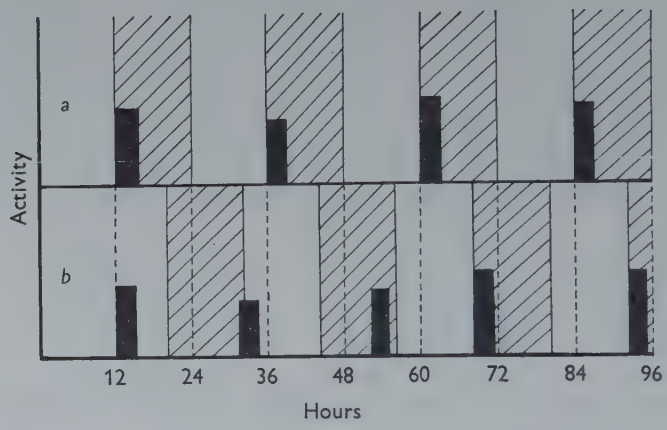


Fig. 8. The peaks of activity of cockroaches when (*a*) onset of darkness occurs at the 'normal' time—control experiment; (*b*) darkness occurs 8 hr. later than normal, new dark:light phases continued for several days.

If the experiment of Fig. 7*b* (unoperated animals) were continued for several days and the episodes of neurosecretion were successively brought forward, this would be equivalent to a shortening of the period of the neurosecretory cycle. The result could be that eventually the onset of darkness would fall within the period of 'possible' secretion. When this occurred there would be an abrupt shift of the activity peak so as to coincide with the onset of darkness. This in fact is observed to happen (Fig. 8).

The effects described above are similar to the transient effects discussed in the Introduction; as was there suggested they appear to have arisen through the interaction of two factors.

(iii) The length of the active period is nearly constant whether 4 or 8 hr. darkness is experienced, except in those cases in which the peak of activity coincides with the time of the inhibitory effect of a change from darkness to light (Figs. 4*d*, 5*d*).

The length of the active period is also the same even when the animal has recently been active, as, for instance, when the dark period begins 4 hr. later than normal (Fig. 6*b*).

These results suggest that either the form of the neurosecretory cycle is not dependent upon the exhaustion of some substance at the end of the secretory period, or that the replacement of the secretory material (or of some precursor) is very rapid.

There is some evidence in support of each of these alternatives. The persistence for some days of a 24 hr. secretory cycle in isolated ganglia points to a certain

independence of the secretory cells. On the other hand, after a change in the environmental cycle the timing of the next episode of neurosecretion depends upon the length of time for which the suboesophageal ganglion has been left with its normal connexions (§ii); this suggests that some other centre may be involved in supplying a substance concerned with secretion, or that the secretory cells are not entirely independent. A study of this problem is in progress.

## SUMMARY

1. The locomotor activity rhythm of *Periplaneta americana* in alternating light and darkness is described as consisting of six stages.

2. The effect on the suboesophageal ganglion neurosecretory cycle of a change from light to darkness at each stage of the locomotor rhythm is described, and three stages in the neurosecretory cycle are recognized.

3. The effect on an established locomotor activity rhythm of a change to darkness at various times of day is described in terms of the immediate reaction of the animal and of the subsequent phase relations of the rhythm.

4. The phases of the activity rhythm are not reset if the environmental change occurs during the active period. The final positioning of the phases, when the onset of darkness occurs during the non-secretory phase of the neurosecretory cycle, is dependent upon the subsequent light conditions; transient activity peaks may appear before the stable position is reached.

5. The dependence of the neurosecretory cells on some other centre for the provision of some secretory substance, or precursor, is discussed.

This work was supported by a special research grant from the Department of Scientific and Industrial Research, which is gratefully acknowledged. I wish to thank Prof. V. B. Wigglesworth for his encouragement.

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# INTERNAL FACTORS CONTROLLING THE SUB-OESOPHAGEAL GANGLION NEUROSECRETORY CYCLE IN *PERIPLANETA AMERICANA* L.

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*Department of Zoology, University of Cambridge*

(*Received* 31 *August* 1959)

## INTRODUCTION

The diurnal locomotory activity rhythm of *Periplaneta americana* is dependent upon the rhythmic secretion of a hormone from the neurosecretory cells of the sub-oesophageal ganglion. The phases of this secretory cycle are set by the stimulus of an environmental change from light to darkness (Harker, 1956, 1958*a*), the beginning of secretion being coincident with the time of the stimulus. When a rhythmically secreting suboesophageal ganglion is implanted into an arrhythmic animal this animal subsequently follows an activity rhythm in phase with the neurosecretory cycle of the implanted ganglion: that is, the ganglion can maintain its neurosecretory cycle independently. It has been suggested, however, that other processes following 24 hr. rhythms may affect the neurosecretory cycle, particularly in the absence of alternating environmental factors (Harker, 1958*a*, *b*). This paper describes the interaction of the neurosecretory cycle and a rhythm associated with the nervous system.

## EXPERIMENTS

The experiments described were designed in an attempt to alter the timing of the phases of the suboesophageal ganglion neurosecretory cycle while leaving unaltered the phases of any other 24 hr. rhythm.

### CHILLING THE SUBOESOPHAGEAL GANGLION *IN SITU*

When cockroaches have been kept at 3° C. for a number of hours the phases of their activity rhythms, after return to room temperature, show a time-lag of a period equivalent to that for which the animals were chilled.

A method has been devised (Brown & Harker, 1960) by which the neurosecretory cells of the suboesophageal ganglion can be chilled *in situ*, without the temperature of other parts of the body being lowered.

The temperature of the suboesophageal ganglia of five rhythmically active cockroaches was lowered to 3° C. for 4 hr.; the ganglia were then dissected out and implanted into arrhythmic animals. All of the implanted animals subsequently showed an activity rhythm in which the phases were delayed by 4 hr. (Fig. 1*b*) relative to those of unchilled animals (Fig. 1*a*). It is concluded that the neuro-secretory cycle is halted by chilling, and starts again when the temperature is raised.

The suboesophageal ganglia of another group of animals were chilled for 4 hr. and the ganglia left in their normal positions. The phases of the rhythms of this group showed no delay relative to their previous settings (Fig. 2*a*). The ganglia from these animals were removed 24 hr. after chilling and implanted into arrhythmic animals; the phases of the rhythms which subsequently appeared in the implanted animals also remained unchanged in relation to those of unchilled animals (Fig. 2*b*). The neurosecretory cycle appears to have returned to its previous setting. These results suggest that there is some factor which can reset the neurosecretory cycle, but which in the first experiment had not had time to act before the ganglia were removed.

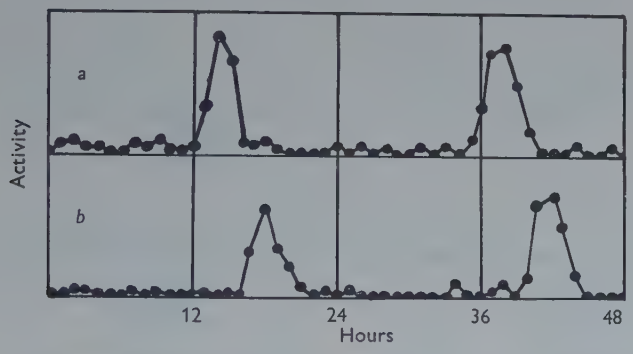


Fig. 1. Activity of previously arrhythmic cockroach into which had been implanted suboesophageal ganglia taken from: (*a*) normally rhythmic cockroaches, (*b*) cockroaches in which the ganglion had been chilled for 4 hr.

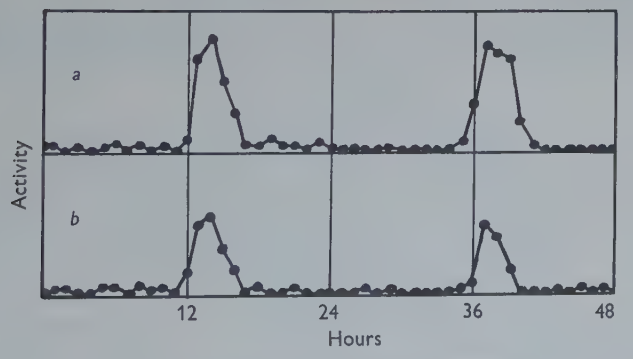


Fig. 2. (*a*) Activity of cockroaches in which suboesophageal ganglia had been chilled for 4 hr.; (*b*) activity of previously arrhythmic cockroaches into which the suboesophageal ganglia taken from (*a*) were implanted 24 hr. after chilling.

These experiments were repeated, the period of chilling being extended to 8 hr. in one group, and 18 hr. in another.

The neurosecretory cycle is delayed by 8 or 18 hr. according to the treatment, as can be shown by implantation experiments.

When a suboesophageal ganglion is chilled for 8 hr. and left with its normal nervous connexions there is a subsequent time-lag of 8 hr. in the phases of the

activity rhythm of the animals (Fig. 3*b*). It appears that whatever factor resets the neurosecretory cycle after it had been delayed by chilling for 4 hr. does not reset the cycle after it has been delayed for 8 hr.

When the activity of the animals is measured after the suboesophageal ganglion has been chilled for 18 hr. two peaks of activity appear in the next 24 hr., the larger occurring 18 hr. later than the peak in the control group, and the smaller about an hour before that of the controls (Fig. 3*c*). The larger peak appears at the expected time in relation to the delayed neurosecretory cycle.

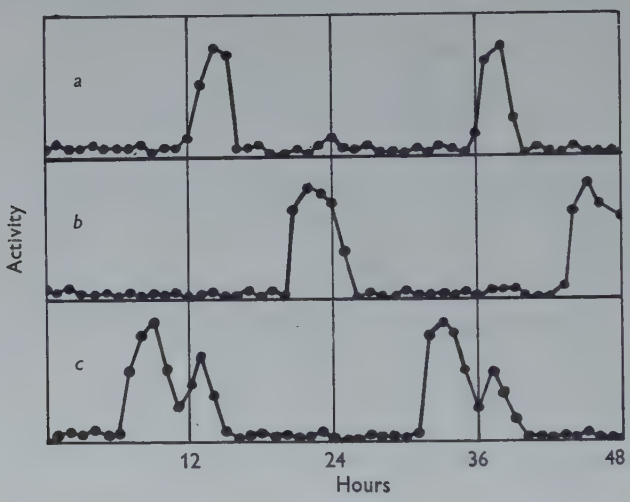


Fig. 3. (*a*) Activity of normal cockroach; (*b*) activity of cockroaches after the suboesophageal ganglia had been chilled for 8 hr.; (*c*) activity of cockroaches after suboesophageal ganglia had been chilled for 18 hr.

It has been shown (Harker, 1960) that there is a stage in the neurosecretory cycle in which secretion does not normally take place, but when it can be induced by a stimulus ('possible' secretion, Figs. 4–6). It can be seen by reference to Fig. 4 that when this stage of 'possible' secretion overlaps with the time during which the animals had begun to be active prior to chilling (as it does after 4 or 18 hr. chilling, Fig. 4*a*, *c*), a peak of activity appears at this time. This represents the only peak after 4 hr. chilling, and the smaller peak after 18 hr. chilling. It is suggested that an internal rhythmical factor, unaffected by chilling of the suboesophageal ganglion, can act as a stimulus on this stage of the cycle. When the neurosecretory cycle has been delayed by 8 hr. the stage of the cycle in which no secretion can take place, even in the presence of a stimulus, coincides with the time at which the animals had been active prior to chilling, and therefore even if a stimulus occurs no peak of activity would be expected to appear (Fig. 4*b*). The results confirm this expectation.

## EFFECTS OF A CHANGE FROM LIGHT TO DARKNESS WHILE THE SUBOESOPHAGEAL GANGLION IS CHILLED

The suboesophageal ganglia of a group of normally rhythmic animals were chilled, beginning 4 hr. before the expected appearance of the activity peak, and continuing for 4 hr. About 15 min. after chilling commenced the compound eyes and ocelli of the animals were blackened with paint. The activity rhythm of the animals was measured after their return to room temperature; the phases showed a 4 hr. time-lag (Fig. 5). In the experiment described in the previous section the chilling of the

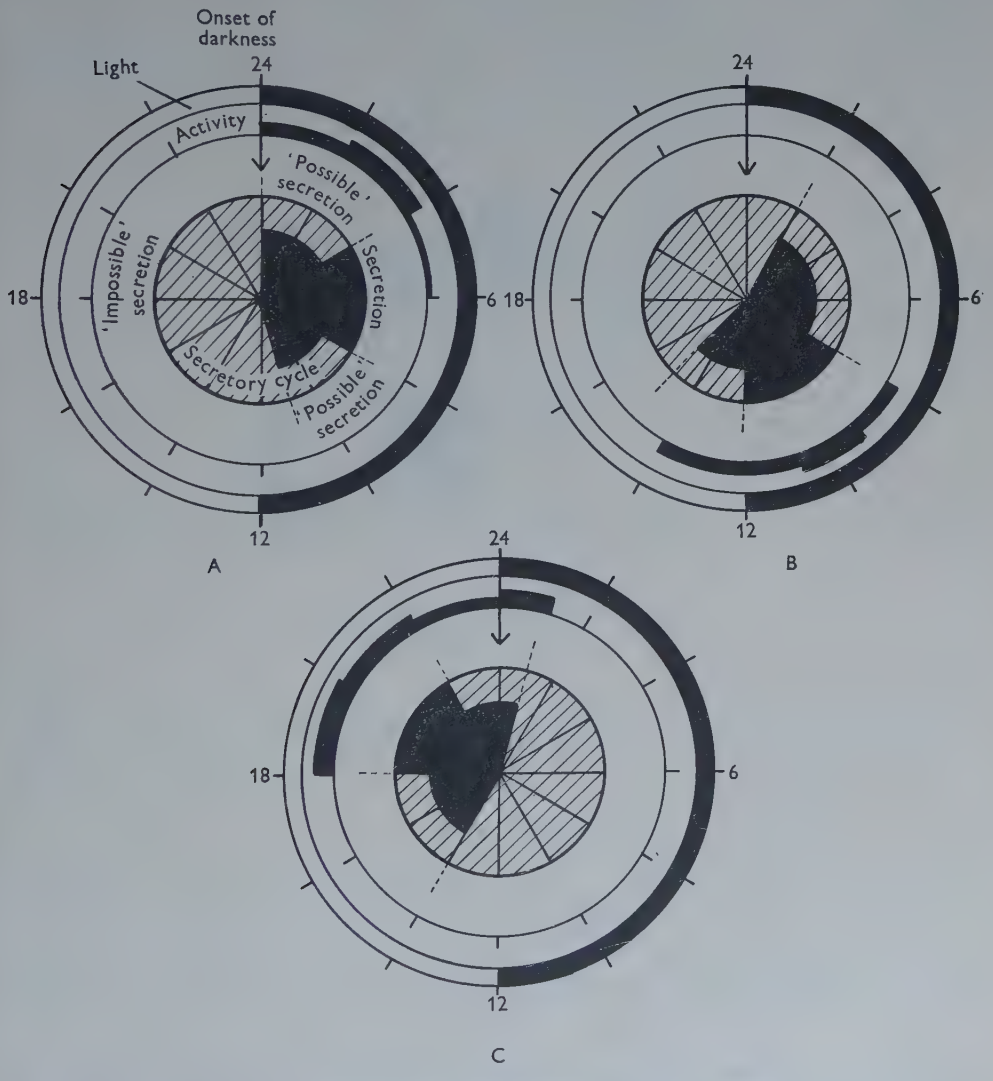


Fig. 4. The activity of cockroaches related diagrammatically to the neurosecretory cycle after the suboesophageal ganglia had been chilled for (A) 4 hr., (B) for 8 hr., (C) for 18 hr.

ganglion for 4 hr. was not followed by a delay in the phases of the rhythm; therefore it appears that in the present experiment the new time of onset of darkness had altered the phases of the factor which stimulates neurosecretion. In order to test this supposition further the ganglia of another group of animals were chilled for 8 hr. beginning just before the activity peak was expected. After 4 hr. of the cold treatment the eyes were blackened. Since it is known from previous experiments that the secretory cycle is delayed for 8 hr. by the chilling procedure, the time when the eyes were covered (onset of darkness) coincides with that part of the neurosecretory cycle in which secretion will take place in the presence of a stimulus. The neurosecretory cells having been chilled at the time of onset of darkness are not directly affected by this stimulus, as was confirmed by implantation experiments. The animal, however, shows an activity peak 24 hr. after the onset of darkness which again suggests that a secondary factor, which can stimulate the neurosecretory cells, has had its phases reset by the onset of darkness.

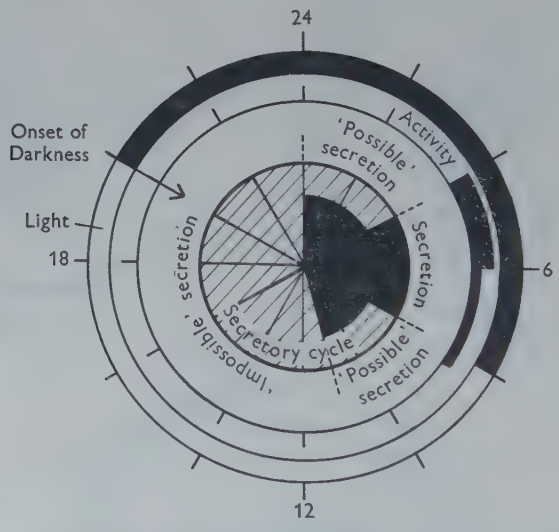


Fig. 5. The activity of cockroaches related diagrammatically to the neurosecretory cycle after the time of onset of darkness had occurred 4 hr. earlier than normal while the suboesophageal ganglia were being chilled for 4 hr.

## DISCUSSION

The results show that although a light-to-darkness change in the environment immediately sets the phases of the secondary rhythm, it does not directly cause the synchronization of the neurosecretory cycle with the environmental conditions. Observations made on the effect of single environmental perturbations on well-established rhythms support this conclusion (Harker, 1960).

The value to the animal of the control of the activity rhythm by two interacting cycles may lie in the fact that the secondary cycle, although itself immediately reset by any change from light to darkness, can only affect the secretory cycle at a time

fairly close to that of the dark period of the preceding days. This allows for the resetting of the activity rhythm by changes in day-length, but not for resetting by light changes at abnormal times of night, unless they recur several times. In view of the small changes in light intensity needed to position the phases of a new rhythm in a previously arrhythmic animal it would appear likely that the change from bright moonlight to darkness would be sufficient to reset the secondary cycle. If the activity rhythm were also reset the animal would soon get out of phase with day and night.

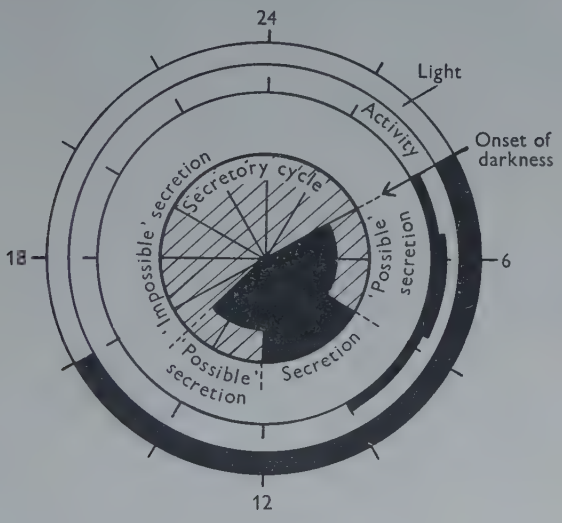


Fig. 6. The activity of cockroaches related diagrammatically to the neurosecretory cycle after the time of onset of darkness had occurred 4 hr. later than normal while the suboesophageal ganglia were chilled for 8 hr.

## SUMMARY

1. Two interacting factors, both following a 24 hr. rhythm, are found to be concerned in the control of the locomotor activity rhythm of *Periplaneta americana.*

2. When the suboesophageal ganglion is chilled to 3° C., the rest of the body being kept at room temperature, the phases of the neurosecretory cycle are delayed for a period equivalent to the period of chilling.

3. A second cycle, which follows a 24 hr. rhythm, can act as a stimulus to the neurosecretory cycle if the latter is at a stage which responds to a stimulus. If the second cycle affects the neurosecretory cycle the phases of the latter are reset by the stimulus.

4. The phases of the second cycle can be reset by a change from light to darkness while the suboesophageal ganglion is in the chilled state. It appears that the second cycle is immediately reset by the onset of darkness, regardless of the time at which this occurs.

5. The value to the animal, in its natural conditions, of the control of the locomotor rhythm by two interacting cycles is discussed.

This work was supported by a special research grant from the Department of Scientific and Industrial Research, which is gratefully acknowledged. I wish to thank Prof. V. B. Wigglesworth for his encouragement.

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# HEAT LOSS AND THE BODY TEMPERATURES OF FLYING INSECTS

## I. HEAT LOSS BY EVAPORATION OF WATER FROM THE BODY

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(*Received* 16 *June* 1959)

(With Plate 2)

### INTRODUCTION

Although an insect's body temperature usually stays near that of its immediate surroundings, three things can cause important departures from it: the absorption of solar radiation, the rapid generation of heat in the muscles during flight, and the removal of heat by evaporation of body water.

Considerable work has been done on the warming of insects by the sun. Mostly the insects were stationary, but Digby (1955) suspended his animals in moving air and his experiments should show the effects of sunshine on the temperatures of insects in flight. Measurements also have been made of the energy released by insects during flight and of their body temperatures in certain situations, and there has been a lot of work on evaporative cooling of inactive insects. But there has been no general study of the dissipation of metabolic heat in flight, no careful investigation of the effects of cooling by evaporation, radiation, and convection on the temperatures of the thorax and other parts of a flying insect's body. The two papers in this series are intended to help fill the gap.

Metabolism during tethered flight is usually between 20 and 50 times and is sometimes as much as 500 times that of resting insects (Weis-Fogh, 1952; Chadwick, 1953; Edwards, 1953; Hocking, 1953; Wigglesworth, 1953). In a large insect the heat generated in flight exceeds that received by the thorax from the sun. The flight muscles of a $1\frac{1}{2}$ or 2 g. insect can be conservatively estimated to produce an average of more than 3 cal. of heat/min. The thorax of an insect this size might present a surface of about 1 cm.$^2$ to the sun. The maximum solar radiation in southern England is about 1·5 cal./min. cm.$^2$ (Pairy, 1951), and the insect's thorax might absorb about 1 cal./min., the rest being reflected from the surface or transmitted through the body. In a small insect, of course, metabolism would be less important than sunshine (Digby, 1955), assuming that the heat generated in flight is more or less proportional to the volume of the flight muscles (see Church, 1959); the small insect would have much less muscle but not so much less surface to intercept the sun's radiation.

The effect of evaporation on the temperatures of inactive, well waterproofed insects is important only in warm, dry, still air. But at 40° C. temperature depressions of 3–5° C. are not uncommon, and at temperatures high enough to cause a partial breakdown in the waterproofing of the cuticle (Wigglesworth, 1945; Holdgate & Seal, 1956; Beament, 1958) evaporative cooling might be greater. Much of the evaporation is through the tracheal system even in inactive insects (Koidsumi, 1934–5; Mellanby, 1934; Wigglesworth & Gillett, 1936), and flying insects breathe much harder and therefore must lose more water. During flight, even at moderate temperatures, it seemed that evaporation might remove a substantial part of the heat generated by the muscles. If so, then the humidity, the insect's permeability and its behaviour, and other factors that affect the rate of evaporation would have considerable effect on the animal's temperature. At the highest temperatures, evaporation was expected to account for most of the heat produced in the flight muscles, although the evidence was inconclusive.

Sotavalta's (1954) measurements of the thoracic temperatures of flying insects suggested that evaporative cooling at high temperatures was important and provided a measure of body temperature regulation. He said, 'In *Bombus* the upper limit [of the temperature excess] at temperatures below 25° is about 16°, and above 35° it is about 4–5°...'; thus the thoracic temperatures remained at about 40° C., whatever the outside temperature. This could be explained by assuming that most of the metabolic heat was 'evaporated' away at 35° C. In other Hymenoptera the effect was less pronounced, for 'in *Polistes* the upper limit is about 5° at temperatures less than 25° and about 4° at temperatures from 35° on'. He concluded tentatively that some species maintain a fairly constant body temperature while flying and seemed to think that they could control either the rate of tracheal evaporation or the rate of heat production, or both, *at will*.

However, there are two reasons for doubting this. First, Sotavalta compared the maximum temperature excesses recorded at 25 and 35° C.; there was much less difference between the average temperature excesses. Second, at 35° C. the flights were all very short and usually the thoracic temperatures were still increasing at the end of them. If the insects had kept flying they might have become much hotter but they stopped flying to keep from doing so. Sotavalta offered this as a possible alternative explanation.

Cooling by convection and long-wave radiation must also be considered. Many medium and large insects produce enough heat while flying, in addition to whatever is lost by evaporation, to warm the thorax 5–15° C. above the temperature of the air (Krogh & Zeuthen, 1941; Sotavalta, 1954; Weis-Fogh, 1956). Whenever an insect's temperature is much different from the surrounding temperature, heat exchange by convection must be important (Gunn, 1942; Parry, 1951; Edney, 1953). Indeed, convection, and perhaps long-wave radiation also, might prove to be more important than evaporation, especially at moderate temperatures. Digby (1955) and Parry (1951) have shown that the wind velocity, for example, greatly affects the temperature excesses of irradiated insects.

My experiments on heat loss in flight by convection and radiation and the in-

sulating effects of air sacs and body hair are described in the second paper. First I had to show how much of a flying insect's heat actually is dissipated by evaporation and to establish whether its equilibrium temperature excess is much smaller in a hot environment than in a cool one.

## METHODS AND APPARATUS

Live, flying specimens had to be used for most of the experiments on the effects of evaporation. The role of evaporation in cooling was most readily determined by recording the effects of changes in the humidity on an insect's pterothoracic temperature. It is, of course, the temperature of the pterothorax that deviates most from that of the environment and is most likely to influence flight. Insects flying steadily in moist air were switched, still flying, to dry air and any decreases in their temperatures were measured.

Unfortunately, these measurements could not be guaranteed free from error. Therefore, the weight of water evaporated from the body during flight also was measured, and the heat necessary for its vaporization was calculated and compared with that generated by metabolism. From this information an estimate was made of the temperature depression to be expected from the water loss.

Whether changes in the insect or its behaviour at high temperatures much affected the rate of evaporation and the animal's temperature excess was best shown by comparing results from flights in very warm and cooler air.

The insects were flown on a rotating mill, impaled on one junction of a thermocouple, inside a cabinet in which the atmosphere was regulated. Each animal was flown at a pair of different temperature-humidity combinations and the results were compared directly. Thus, there was no need to use very large numbers of insects to compensate for individual variations in size and flight characteristics, and specimens that behaved poorly could be discarded without biasing the results.

### *The flight mill and cabinet*

The plywood cabinet was waxed on the inside and insulated with a double layer of corrugated cardboard (Pl. 2, fig. 1). It was about 1 m. wide and $\frac{1}{3}$ m. deep. There was a removable Perspex window in the top. Air was drawn by a fan through an external conduit at the bottom of one side. Baffles at the entrance and exit of the conduit broke up the air stream and directed it away from the insect's flight path. In the conduit were a 400 W. heater connected to a variable transformer and either a simple wet wick humidifier or silica gel drier (Pl. 2, fig. 1).

The air in the cabinet could be raised or lowered through 10° C. and moistened to over 95 % R.H. or dried to about 40 % in about 10 min. The temperature could be kept constant within $\pm 0{\cdot}1$° C., and with a large insect stirring up the air it was sufficiently uniform throughout the cabinet.

The flight mill was pivoted on a stand in the centre of the cabinet (Pl. 2, fig. 1). One type of mill, used with desert locusts, is shown in Pl. 2, figs. 2 and 3. The insect was impaled on one junction of the copper-constantan thermocouple carried by the supporting arm. The wires from this thermojunction, and the insect, were

supported by a glass tube fastened to the end of the arm. The tube also insulated the wires against heat loss. The reference thermojunction of the couple was in front of the insect, embedded in a small plasticine ball. One end of the flexible steel-wire supporting arm was bent sharply down for an axle, which was supported by a capillary tube embedded firmly in a cork disk. The sharpened end of the axle turned on a piece of glass at the bottom of the capillary. The cork disk was set in the top of a metal cylinder sunk into the cabinet floor. The free ends of the 40-gauge insulated copper wires from the thermocouple dipped into the mercury in a pair of concentric cups set in the cork around the axle. Heavier wires led from the mercury through the cabinet floor to an ammeter. The mercury contacts created extraneous thermojunctions, but so long as the two cups of mercury were of uniform temperature they introduced no error, and the temperature of the mercury became uniform soon after each change in air temperature. The thermocouples were calibrated, accurate to 0·1° C., for several representative reference temperatures.

Krogh (1948) noted that large errors can arise from heat being conducted away from the insect through the thermojunction leads. First, the thermojunction may lose heat to the wires faster than it can absorb it from the insect unless there is an appreciable temperature gradient between the junction and the insect's tissues. Enlarging the thermojunction helps. Second, the entire insect may be cooled faster than normally. In both cases insulating the wires as Krogh did will help.

First, a 0·7 or 0·8 mm. bead or dart of solder was applied to the 'insect' thermojunction. From this the lacquered wires were passed through two concentric glass tubes, separated by an air space. The inner tube extended back from the bead and the outer tube began 7 mm. from the bead and covered the rest of the inner one. The glass tubes were fixed to the wires by only a small drop of cement at each end so not to destroy the insulating air spaces. If the insulated thermojunction was inserted less than 2 mm. into an insect it was not reliable, but so long as it was in at least 4 mm. it measured the actual temperature of the tissues accurately, even in a wind of more than 300 cm./sec. Ordinarily it was inserted 7 mm. into the locust. In a 200 or 300 cm./sec. wind less than 2% of the heat produced by a locust was lost through the wires and insulation. With a smaller insect and the same thermojunction assembly, of course, the error would be relatively larger.

### *Estimation of flight intensity*

The temperature excess of the thorax depended primarily on the intensity of flight and the rate of heat production, so a measure of any changes in flight intensity during an experiment was needed if the influences of other factors on the temperature excess were to be demonstrated.

The speed of flight was easily measured by counting the number of revolutions of the mill each minute. In an individual measurement the maximum error was about 5 cm./sec., which would be represented by a rise or fall in thoracic temperature of 2–4%, generally equivalent to 0·2° C. or less. But the averages of several measurements were used and these would be more accurate.

However, the main component of an insect's flight is lift (Jensen, 1956; Weis-

Fogh, 1956). This was judged from the sag of the loaded end of the flight mill arm. A vertical scale of alternate black and white lines, each 2 mm. wide, was fastened to the inside of the cabinet (Pl. 2, fig. 1), and a narrow mirror was attached to the thermojunction holder just below the insect (Pl. 2, fig. 3). As the animal flew by, the height of the mirror could be seen against the scale through a hole in the opposite side of the cabinet. The lift generally could be determined within 5%, and the temperature excess was roughly proportional to the lift.

### *The experimental insects*

Migratory-phase desert locusts, *Schistocerca gregaria* Forsk., which are large, persistent, steady fliers, were used in the main experiments. Their metabolic rate and the kind of fuel they consume in flight were already known (Weis-Fogh, 1952). This information was needed for the check experiments, in which the effect of evaporation on the temperature was estimated from the rate of water loss.

Only mature males were used; the females were usually too heavy to fly well. Most of the insects were generously supplied as young adults by the Anti-Locust Research Centre, London. They were then reared to maturity on wheat sprouts in a warm cage. A week or so before they were to be used their hind tibiae and mid-tarsi were amputated (Krogh & Weis-Fogh, 1952) to prevent them from grasping the thermojunction holder for support.

### *Mounting the insects on the flight mill*

A locust was anaesthetized with $CO_2$ and the thermojunction inserted slightly off-centre into the ventral surface of the thorax. The bead was pushed up into the dorso-medial part of the pterothorax, near the longitudinal flight muscles. A pair of wire struts with small plastic tips (to reduce heat conduction) attached to the thermojunction holder supported the outside corners of the pterothorax (Pl. 2, fig. 3). The locust was stuck to the strut tips and the thermojunction sealed in place with beeswax and resin (Krogh & Weis-Fogh, 1951). The insect was set at a small angle with the horizontal to simulate its normal flight posture. When it had recovered from anaesthesia it was easily started flying by removing from its grasp the small wad of paper it had been allowed to hold.

### *Selecting comparable periods of flight*

The intensity of flight reached a peak in 5 or 6 min. Then, in the next hour, the speed decreased from about 350 cm./sec. to less than 300, and the thoracic temperature excess dropped about 3° C. There was also a decrease in the ratio of lift to thrust, and flight often was irregular. But during the second hour the better fliers steadied and then flew evenly for long periods, unless they were disturbed (cf. Weis-Fogh, 1952, 1956).

Each locust was first flown for about 1½ hr. at 30°. Then the temperature and humidity were adjusted to the appropriate values, and when the insect's flight and temperature had stabilized, records were made every 2 min. of its speed, lift, and temperature excess and of the air temperature and humidity. After about ½ hr. the

humidity was altered—in the first series of experiments—and the process repeated. The air was alternately moistened and dried as long as the insect continued to fly fairly steadily at 260 cm./sec. or more and supported at least 70 % of its own weight.

Data were collected from a fairly large number of locusts flown at various temperatures, alternately in moist (95–99 % saturated) and in drier (35–45 % saturated) air, and then these were searched for usable parts. Wherever an insect's performance was the same during an interval of 12–14 min. in each of two successive periods, one moist and one dry, the readings taken during those intervals were averaged and compared. The temperature readings were hidden while the useful intervals were selected so that they would not influence the decisions. I tried to get as many pairs in which the dry period preceded the moist as the reverse, to eliminate the effect of any undetected decrease in the ratio of lift to speed that may have persisted beyond the preparatory flight. A difficulty was that many of the insects flew less readily in dry air; though an insect might provide a long series of otherwise good readings, there were often no two successive dry and moist sets at comparable intensities.

## COOLING BY EVAPORATION IN *SCHISTOCERCA* AT 30, 35, AND 40° C.

Few of the locusts could fly effectively for more than a few minutes at 25° C. But at 30° C. good fliers often kept going strongly and steadily for 6 or 8 hr. and at 35° C. flights were at least as strong as at 30° C., though not quite so long. At 40° C., although some specimens flew steadily for 2 or 3 hr., many would not fly for more than 10 or 15 min. and, if forced to keep trying, became paralysed. At 45° C. continuing flight was impossible in all cases; rarely did a locust reach its potential maximum temperature excess before it weakened and stopped.

At 30, 35, and 40° C., thirty-three successful flights were obtained. In most of the flights the speed was between 270 and 300 cm./sec. and the lift between 70 and 100 % of the insect's weight. The average performance was only about 15 % below normal. (When mounted in a wind tunnel to simulate natural flight very closely, *Schistocerca* averages about 350 cm./sec. and produces 100 % lift (Weis-Fogh, 1956).) The drag of the mill was almost equal to that of a locust, but that amount of extra drag has very little effect on the speed of flight (Krogh & Weis-Fogh, 1952; Weis-Fogh, 1956).

The results of the flights (Table 1) indicate that the dissipation of heat by

Table 1. *Effect of evaporation on the temperature excess of flying* Schistocerca *at various air temperatures*

| No. of flights | Air temperature (° C.) | Average vapour pressure difference between moist and dry air (mm. Hg) | Average temperature excess (° C.) | | Temperature excess difference (° C.) |
|---|---|---|---|---|---|
| | | | In moist air | In dry air | |
| 14 | 30 | 18 | 6·2 | 6·0 | −0·2 (±0·05) |
| 10 | 35 | 24 | 5·9 | 5·5 | −0·4 (±0·05) |
| 9 | 40 | 29 | 6·3 | 5·8 | −0·5 (±0·08) |

evaporation is not very important. Large changes in the humidity of the air have relatively little effect on a flying locust's temperature excess.

At 40° C. an effective cooling mechanism would have been of considerable advantage to the insects, but there was no sign of any special mechanism operative at 40° C. during flight. Breathing seemed no more vigorous than at 30 or 35° C. Moreover, the temperature measurements showed that the rate of cooling per unit vapour pressure deficit was probably not much greater at 40° C. than at 30° C. It is significant also that many of the insects had a decided preference for the moist atmosphere, especially at 40° C.

If the 'dry' air had been perfectly dry, of course, the recorded decreases in the temperature excess would have been larger. The *total* decreases produced by evaporation would have been larger yet, because there must have been some evaporation from the warm insects even in the saturated air. Ramsay (1935*a*, *b*) showed that evaporation is best regarded as being proportional to the difference between the saturated vapour pressure at the temperature of the evaporating surface and the actual vapour pressure of the air around it. The relative rates of evaporation from different parts of the body (discussed in a later section) and the temperature distribution in the insect (Church, 1959) were taken into account, and the average effective temperature of the evaporating surfaces of *Schistocerca* was estimated to be about 4° C. above air temperature—or 44° C. in 40° C. air. The vapour pressure difference between air saturated at 44° C. and completely dry air is 68 mm. Hg. The total reduction in temperature excess in perfectly dry air, if proportional to the vapour pressure difference, would then have been $68/29 \times 0{\cdot}5 = 1{\cdot}1$ or 1·2° C. This is still not a large fraction of the temperature excess. At 30° C. the equivalent reduction would have been only about 0·5° C.

## TEMPERATURE EXCESS OF *SCHISTOCERCA* IN DRY AIR AT 28 AND 37° C.

In another set of experiments the humidity was kept at 40–45 % and the air temperature was raised from 28° to 37° C.—in half the experiments—or lowered from 37 to 28° C. At each temperature a series of 10 readings 2 min. apart were taken, beginning 25 min. after the required air temperature had been established. The insects were first given preliminary flights at 30° C., as before. If an insect's speed varied more than 20 cm./sec. during a series of readings, or if it did not fly at least 260 cm./sec. and support 75 % of its weight, it was discarded.

The object was not, as in the preceding experiments, to compare successive periods flown at the same intensity but to demonstrate the effect of the insects' behaviour on the temperature excess. Nearly all specimens flew harder in the warmer air. In addition to a large transient increase in intensity, there was a fairly persistent smaller effect, which lasted at least 45 min. (It might not have been so pronounced over several hours; Weis-Fogh (1952) found that the intensity of prolonged flights was independent of the air temperature between 25 and 35° C.)

During the periods recorded the average speed was about 5 % higher at 37 than at 28° C. (Table 2). An increase of 7–10 % in the temperature excess, or about

0·4–0·6° C., would ordinarily accompany a 5 % increase in flying speed (the temperature excess was generally proportional to the velocity to a power of $1\frac{1}{2}$ or 2). This was probably enough to compensate for the increase in the rate of evaporative cooling. Thus the average temperature excess at 37° C. was very nearly the same as at 28° C. (Table 2).

Table 2. *Comparison of the temperatures of locusts flying in dry air at 28 and 37° C.*

| Air temperature 28° C. | | Air temperature 37° C. | | | |
|---|---|---|---|---|---|
| Average flying speed (cm./sec.) | Average temperature excess (° C.) | Average flying speed (cm./sec.) | Average temperature excess (° C.) | Difference in speed (cm./sec.) | Difference in temperature excess (° C.) |
| 260 | 4·9 | 295 | 6·3 | 35 | 1·4 |
| 260 | 5·2 | 285 | 6·1 | 25 | 0·9 |
| 300 | 6·9 | 315 | 7·5 | 15 | 0·6 |
| 270 | 6·3 | 295 | 6·8 | 25 | 0·5 |
| 290 | 5·4 | 310 | 5·8 | 20 | 0·4 |
| 275 | 7·1 | 290 | 7·3 | 15 | 0·2 |
| 290 | 4·8 | 305 | 5·0 | 15 | 0·2 |
| 290 | 6·9 | 300 | 7·0 | 10 | 0·1 |
| 265 | 5·9 | 270 | 5·9 | 5 | 0·0 |
| 290 | 6·1 | 285 | 6·0 | −5 | −0·1 |
| 270 | 5·8 | 295 | 5·5 | 25 | −0·3 |
| 265 | 5·9 | 270 | 5·6 | 5 | −0·3 |
| 275 | 6·1 | 280 | 5·8 | 5 | −0·3 |
| 315 | 8·6 | 320 | 8·2 | 5 | −0·4 |
| 345 | 7·8 | 340 | 6·8 | −5 | −1·0 |
| Mean | — | — | — | +13 (±3) | +0·1 (±0·15) |

*Schistocerca* obviously does not regulate its body temperature during flight by reducing its metabolic rate (even within the limits that continued flight would permit) when the air is very warm—rather the reverse. In many other insect species there are greater increases in flight intensity with temperature than in *Schistocerca* and such body temperature regulation is out of the question.

## EFFECT OF EVAPORATION ON THE MAXIMUM TEMPERATURE EXCESS IN *SCHISTOCERCA*

Sotavalta (1954) based his conclusions on the *maximum* excess temperatures obtained at various air temperatures. For this reason a comparison of such records obtained with *Schistocerca* is of interest. Maximum body temperatures were generally reached in the first 5 or 6 min. of flight. A fairly large number of records were accumulated from trial flights in moist and moderately dry air at various temperatures. They varied a great deal and only a very rough comparison is justified.

At the highest air temperatures the average maximum temperature excess seemed to be about $\frac{1}{2}$ or $\frac{3}{4}$° lower in dry than in moist air. But even this rather small effect could not definitely be attributed to evaporation, because the maximum intensity of flight also seemed to be slightly lower in dry air.

Sotavalta thought it most likely that the temperature excesses he recorded at 35° C. and above were reduced because of evaporation. The behaviour of *Schistocerca*, however, indicates that the alternative explanation is the better one: that the temperature excesses were small because the insects stopped flying before they overheated. At 40° C., well adapted *Schistocerca* produced maximum excess temperatures about as high as the highest produced at lower air temperatures. Other records were considerably lower, obviously because the locusts did not keep flying long enough to reach their potential maximum temperatures or because their flight was slow or unsteady. At the other extreme, 25° C., maximum excess temperatures also were subnormal and for the same reasons.

## COOLING OF THE PTEROTHORAX ESTIMATED FROM MEASUREMENTS OF WATER LOSS

If one knows how much water an insect loses in flight, how much of it evaporates from the pterothorax, and how much heat the flight muscles produce, one should be able to estimate the effect of evaporation on the insect's temperature excess. This approach was used to check the temperature measurements described above. The heat generated in flight by *Schistocerca* was already known (Weis-Fogh, 1952), but the water loss had to be measured.

The total water loss was easily determined. Although *Schistocerca* uses 1 % of its body weight in fuel per hour during steady flight, after the first hour or so it burns only fat (Weis-Fogh, 1952), and each milligram of fat produces an equal weight of water when oxidized. Thus the weight loss (excluding faeces) after the first hour represents evaporation.

The insects were flown in the cabinet on a mill with a removable mount for 1½ hr. at 30° C. Then each insect's mouth and anus were sealed with wax to prevent any excretion, and the weight losses during several successive ¾ or 1 hr. flights at about 40 % R.H. and 30 and 40° C. were measured. Only data from specimens that maintained the same strength of flight as did those in previous experiments were used. There seemed to be no systematic change in the rate of weight loss during the periods recorded. Control flights in moist air confirmed that the weight losses were due essentially to evaporation.

Table 3. *Total rates of water loss from* Schistocerca *during flight in dry air*

| Air temperature (° C.) | Vapour pressure difference (mm. Hg) | Water loss (mg./locust/hr.) |
|---|---|---|
| 30 | 27 | 21 (±1) |
| 40 | 46 | 44 (±2) |

The average water losses from large male locusts (weight about 2 g.) are shown in Table 3. Weis-Fogh (personal communication) also has measured the evaporation from *Schistocerca* flying at 30° C. in connexion with his analysis of flight, and our results were similar.

The differences in vapour pressure between the evaporating surfaces of the insects and the air, shown in the second column of Table 3, are based on the assumption that the effective average temperature excess of the surfaces was about 4° C. On that basis the total rate of evaporation per unit vapour pressure difference was only about ¼ greater at 40 than at 30° C.

If the figures in Table 3 are extrapolated to 0% R.H., *Schistocerca* would lose about 31 mg. of water per hour at 30° C. and 65 mg. at 40° C. The heat of vaporization of water at such temperatures is about 575 cal./g. The total amount of heat dissipated by evaporation in dry air at 30° C. would be about 0·30 cal./min. and at 40° C., 0·62 cal./min. Weis-Fogh (1952) has established the metabolic rate of a 2 g. locust as 2·5 cal./min. The power output amounts to only 10–20% of this (Jensen, 1956), so about 85% of the energy must be released in the flight muscles as heat. If on the average my insects flew at 85% of normal strength, they probably produced about 1·8 cal. of heat/min. According to these figures, when the air is completely dry at 30° C. evaporation would dissipate 17% of the heat produced and at 40° C., 35%.

However, only the heat lost by evaporation from the pterothorax—more strictly, only that from the air sacs and tracheae among the flight muscles—will have a direct effect on the pterothoracic temperature excess. Its effect will be directly proportional to the relative amount of energy involved; in a 300 cm./sec. wind the temperature excess of an internally heated locust (see Church, 1959) with its spiracles sealed was shown to be proportional to the rate of heat production, except for a reduction of 1 or 2% in the temperature excess as it approached 10 or 15° C. The evaporation from the pterothorax and that from the rest of the body could not be measured directly, but it was possible to differentiate between the evaporation from the tracheal system and that through the cuticle, which proved almost as useful.

Transpiration through the cuticle was measured under the same conditions as the measurements of total water loss. The locusts were rotated on a mill in the cabinet at 275–300 cm./sec. at about 40% R.H. Their wings were extended and the mouth, anus, and spiracles sealed. Because the average surface temperature of a flying locust is roughly half a degree above air temperature, exposures were made at 30·5 and 40·5° C. Many of the insects asphyxiated during the drying, but there seemed to be no consistent change in the rate of evaporation. At 30·5° C. the average water loss was $7 \pm 0{\cdot}6$ mg./hr. for a 2 g. locust and at 40·5° C. it was $17 \pm 1$ mg./hr. These values were equivalent to roughly one-third of the total water losses.

The area of cuticle covering the pterothorax is only a small part of the total area. So, of the third of the evaporation that is external, most must occur some distance from the flight muscles. Relatively little heat is conducted from the pterothorax to the other parts of the body (Church, 1959), and therefore most of the cuticular evaporation can have but little effect on the pterothoracic temperature. Similarly, water that evaporates in the abdominal tracheae will have little effect. However, of the two-thirds that is lost through the tracheal system most undoubtedly evaporates

in the pterothorax. According to Weis-Fogh (1953) the rapid contractions of the pterothorax during flight are responsible for most of the tracheal ventilation—abdominal movements play relatively little part during flight—and most of the dry air from outside enters the front of the insect and will become saturated in the pterothorax (Weis-Fogh, personal communication). (Apart from evaporation, the heat removed by the air itself amounts to less than 1 % of the metabolic heat.)

Thus, probably about two-thirds of the total evaporation is important in cooling the pterothorax. In perfectly dry air, then, one would expect a total reduction in the temperature excess of about 11 % at 30° C. and 23 % at 40°, or reductions of 0·7 and 1·4° C., respectively, from a temperature excess of 6° C. These figures are close to those derived in an earlier section from the temperature measurements.

It is clear why increases in the permeability of the cuticle produced by raising the temperature have so little effect on a flying locust's temperature excess. At ordinary temperatures there is so little evaporation through the cuticle from the pterothorax that it would have to increase many times before it became important. Measurements similar to Holdgate's (1956) showed that the cuticle of *Schistocerca* is only about twice as permeable at 45° C. as it is at 30° C. *Schistocerca* stops flying well before it gets hot enough for evaporation through the cuticle to be of much use.

## COOLING BY EVAPORATION IN *TRIPHAENA* AND *BOMBUS* AT 28° C.

Desert locusts are very well waterproofed, but they are not unique. According to some rough measurements, the rate of evaporation through a square centimetre of cuticle at 30° C. was not notably different from the rates in the moths and bees referred to below, nor in other efficiently waterproofed insects. Neither did it seem likely that the air sacs and tracheae of *Schistocerca* are much less permeable than those of other terrestrial insects. But even if they are, few insects from more temperate climates can keep flying at such high temperatures as *Schistocerca* and it seemed unlikely that evaporative cooling at the highest temperatures they could endure would be much more important than it is in *Schistocerca* at 40° C. To check this hypothesis temperature measurements also were made on some large noctuid moths, *Triphaena pronuba* Kubb., and on queen and large worker bumble bees, *Bombus lapidarius* Linn.

The insects were flown in the cabinet on a very light-weight mill. The thermojunction was inserted in the centre of the scuto-scutellar suture of the mesonotum in the moths, and in the pronotum, offset slightly and projecting back at an angle in towards the centre of the pterothorax, in the bumble bees.

The moths were field-collected and kept in the laboratory up to 4 days, fed on sugar and water. Mostly males were used but the females were as good, if not heavily laden with eggs. They usually flew better at night in the dark, and a little more persistently in moist than in dry air, but probably not any harder.

The strength of flight in *Triphaena* was about the same from 20 to 30° C., though the moths were easier to start at the higher temperatures and needed no warm-up. A good flight would last well over an hour at 20 or 25° C. and about half as long at

30° C. At 35° C. they would start off strongly but would stop after only a few revolutions.

A series of flights were made at 28° C. Each insect was flown for two successive intervals, one in moist air and one in fairly dry air, with a rest between. In half the flights the moist interval was first. During the first 10 min. of each interval no body temperature measurements were recorded. During this time the temperature excess rose to a peak, dropped a little, and began to level off. For the next 8 min. the speed and temperature excess were recorded each minute and the figures averaged. The flight intensity and temperature excess often fluctuated considerably but the mean values are probably reliable. No attempt was made to measure lift, but the insects generally seemed to support most of their weight. A flight was abandoned if the speed dropped below 180 cm./sec. No abdominal respiratory movements could be seen.

Table 4. *Effect of evaporation on the temperature excess of* Triphaena *flying at an air temperature of* 28° C.

| In moist air (96–100 % R.H.) | | In dry air (35–50 % R.H.) | | | |
|---|---|---|---|---|---|
| Average flying speed (cm./sec.) | Average temperature excess (° C.) | Average flying speed (cm./sec.) | Average temperature excess (° C.) | Difference in speed (cm./sec.) | Difference in temperature excess (° C.) |
| 220 | 7·3 | 180 | 5·1 | −40 | −2·2 |
| 225 | 10·9 | 220 | 9·4 | − 5 | −1·5 |
| 210 | 6·7 | 180 | 5·3 | −30 | −1·4 |
| 255 | 7·6 | 235 | 6·3 | −20 | −1·3 |
| 285 | 10·7 | 265 | 9·5 | −20 | −1·2 |
| 250 | 9·7 | 230 | 8·5 | −20 | −1·2 |
| 180 | 8·3 | 185 | 7·2 | + 5 | −1·1 |
| 190 | 7·0 | 185 | 6·1 | − 5 | −0·9 |
| 200 | 5·7 | 200 | 5·0 | 0 | −0·7 |
| 180 | 6·3 | 190 | 5·8 | +10 | −0·5 |
| 255 | 8·8 | 275 | 8·5 | +20 | −0·3 |
| 205 | 5·7 | 215 | 6·1 | +10 | +0·4 |
| 265 | 9·6 | 290 | 11·0 | +25 | +1·4 |
| 230 | 6·7 | 260 | 8·1 | +30 | +1·4 |
| Mean | — | — | — | − 3 (±6) | −0·7 (±0·3) |

The average flying speed was nearly the same in either atmosphere (Table 4). The average difference in temperature excess, which must indicate the decrease caused by the extra evaporation into the drier air, was small compared with the temperature excess itself. The air temperature was about the maximum that would permit sustained flight; nevertheless a large reduction in humidity caused a temperature drop of less than 1° C.

No way was found of inducing the bumble bees to fly more than a few minutes at a time. Many stopped while the thoracic temperature was still rising, and struggled to get loose. They flew more readily and a little harder at 25 and 30° C. than at 20° C. At 20° C. they usually exercised before taking off, vibrating their wings and pumping the abdomen. At 35° C. flights began strongly but lasted less

than a minute. Strong abdominal breathing movements were maintained during flight; they appeared to be a little stronger at the higher temperatures.

A series of bees were flown at 28° C., each one in moist and in fairly dry air with a $\frac{1}{4}$ hr. rest between. The thoracic temperature was read only once, exactly 4 min. after the flight started and when the temperature excess was approaching its maximum. The insects were all started with body temperatures very nearly equal to the air temperature. Those that stopped in the first 4 min. were not considered.

The range of excess temperatures at 4 min. was 7–16° C., the large queens tending to be warmest. In 17 'successful' flights the average temperature excess in moist air was 10·5° C. In 'dry' air it was $0{\cdot}4 \pm 0{\cdot}6$° C. lower. Not all the flights were smooth, short though they were, and there was no assurance that the insects released the same amount of energy under the two conditions. Nevertheless, the fact that there was relatively little difference between the average excess temperatures in moist and fairly dry air supports the idea that evaporative cooling during flight is not of great importance.

## COOLING BY EVAPORATION IN SMALL INSECTS

Evaporative cooling may be more important in smaller insects, but it is not likely to be so. The rate of evaporation is partly dependent on the insect's surface area, and if the heat produced in flight is more or less proportional to the insect's volume, then evaporation would account for a larger fraction of a small insect's heat. But evaporation is dependent also on the rate of tracheal ventilation, which must be governed largely by the need for oxygen, which in turn depends on the rate of metabolism. Thus the fraction of the heat dissipated by evaporation would tend to be independent of the insect's size. The effect on the insect's temperature excess of both the heat produced and the part lost by evaporation will depend on the rate of cooling by convection and other means. In a very small insect neither the flight metabolism nor evaporation can alter the body temperature very much because a small body is cooled very fast by convection (Church, 1959). The water evaporated in flight by Hocking's (1953) *Drosophila* may have dissipated as much as 60% of the heat the flies produced (though it may have been much less), but it could not have greatly affected their temperatures.

## SUMMARY

1. Comparative measurements of body temperatures and water loss in *Schistocerca gregaria* showed that evaporation dissipates relatively little of the heat generated by the wing muscles during flight.

2. In perfectly dry air at 30° C., evaporation reduces the temperature excess of the pterothorax by less than 10%, or about 0·5° C. Even at 40° C., which is the highest temperature that will permit continuing flight, the reduction is only about 20%, or 1·2° C., in dry air.

3. A flying locust has no special mechanism, except cessation of flight, to protect it from overheating. Breathing is not markedly increased at high temperatures, nor is the rate of heat production reduced.

4. Very little heat is dissipated from the pterothorax by evaporation through the cuticle. The cuticle becomes permeable enough to allow substantial cooling only at temperatures well above the highest that permit flight.

5. Temperature measurements in *Triphaena pronuba* and *Bombus lapidarius* supported the idea that evaporative cooling during flight is not much more important in other well-waterproofed insects. Large changes in the humidity produced changes of less than 1° C. in the temperature excess, even at the highest air temperatures at which the insects could fly.

6. The reactions of the insects to moist and dry air are adapted to the conservation of their water rather than to rapid cooling.

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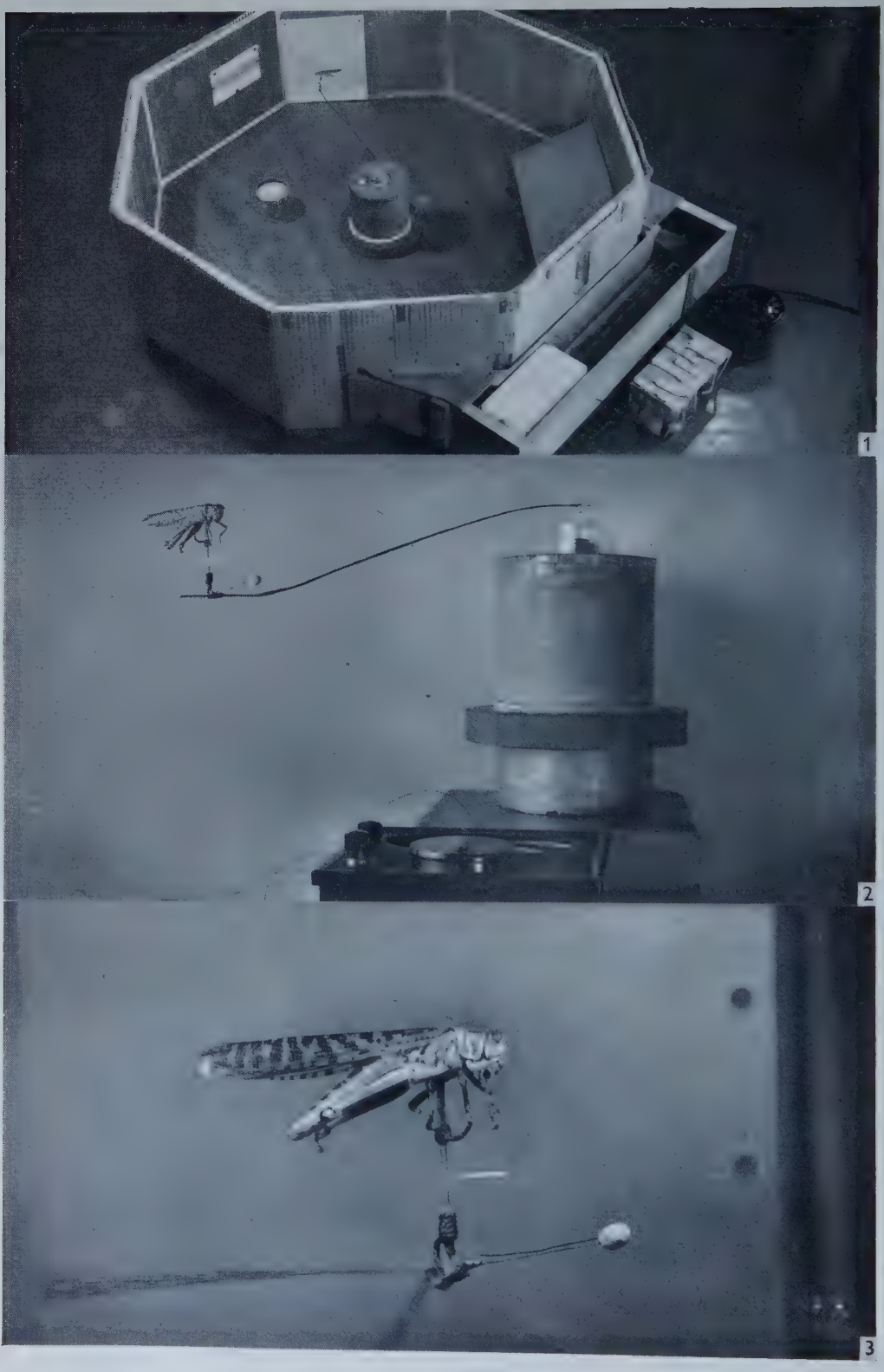


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## EXPLANATION OF PLATE

Fig. 1. Flight cabinet. Top removed to show flight mill; top of conduit (lower right) removed showing heater and drier in place.

Fig. 2. Desert locust mounted on flight mill.

Fig. 3. End of flight mill arm with mounted locust, showing glass support and insulator tube, supporting struts, mirror, and reference thermojunction in plasticine ball.

# HEAT LOSS AND THE BODY TEMPERATURES OF FLYING INSECTS

## II. HEAT CONDUCTION WITHIN THE BODY AND ITS LOSS BY RADIATION AND CONVECTION

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(With Plate 3)

### INTRODUCTION

Evaporation of body water has been shown to have little effect, ordinarily, on the temperature of an insect in flight (Church, 1959). Estimates of long-wave radiation from the body would suggest that it also has little effect. Convection, therefore, promises to be the most important cooling mechanism.

Digby's (1955) is the only analysis of convective cooling in insects, although the principles are well established for objects of regular shape (see Fishenden & Saunders 1950, for example). A detailed discussion of convection is better left for the section in which my experiments are described; however, the important factors are the air speed, the size of the body and the nature of its surface, and its temperature excess. If all the heat from a small, smooth body in a laminar air stream is dissipated by forced convection, the rate of heat loss per square centimetre per degree temperature excess is roughly proportional to the square root of the air velocity over the square root of the body's diameter. Digby found a certain amount of agreement between his irradiated insects and regularly shaped bodies. However, the size of the insects was a more important factor in some cases than was expected.

Heat exchange by radiation is governed by the Stefan–Boltzmann law. The net transfer of heat, $H$, in cal./min. cm.$^2$, from a small body to its surroundings by long-wave radiation is equal to $E \times 8{\cdot}22 \times 10^{-11} (T_1^4 - T_2^4)$, where $T_1$ and $T_2$ are the temperatures in ° K. of the radiating body and of its surroundings, respectively, and $E$ is the emissivity of the body expressed as a fraction of the emissivity of a perfect radiator (Fishenden & Saunders, 1950). The difficulty is in knowing just what values to put into the formula, particularly when the insect is outdoors. Although the amount of heat a flying insect loses by radiation is probably not large, it is not always insignificant.

Whichever way the heat finally escapes—whether by convection or radiation (or by evaporation from the surface) it must first get from the flight muscles to the surface of the body. The relationship between the internal and surface temperatures will not be the same when the heat is generated in the muscles as when, as in Digby's

(1955) experiments, its source is outside. Relative changes in the temperature excess at a point inside a flying insect will not, by themselves, give a fair idea of changes in the rate of cooling at the surface by convection and radiation. The effect of the circulation of the haemolymph has to be considered; it may considerably speed up the transfer of heat from the interior to the surface and to the extremities of the body or it may be too sluggish to have much effect.

There are two kinds of structures in insects that would be expected to insulate the body, particularly the flight muscles, against the rapid loss of metabolic heat. Subcutaneous air sacs would hinder conduction to the surface of the body, and superficial hair and scales—possessed in abundance by hawk moths, noctuids, and bumble bees, for example—would interfere with convection. In some insects at least, the influence of these structures promised to be significant. Digby (1955), however, notices no difference between pubescent and naked species of Diptera and Hymenoptera irradiated in a light wind.

My experiments on the conduction of heat inside the body are discussed first. Temperature gradients in dead, artificially heated insects are compared with those in live, flying specimens. Sample calculations of the heat lost by radiation are then compared with results derived from temperature measurements. In the last section experiments on convective cooling of internally heated insects are described, and the effects of the factors governing convection on the thoracic temperatures of 'flying' insects are demonstrated. The quantitative effects of air sacs and hair are discussed in the sections on internal conduction and convection.

Interpretation of the results proved to be relatively easy because of three things. The first was that the over-all rate of cooling of a warm insect under constant external conditions is usually very nearly proportional to its temperature excess; the extent of any experimentally induced increase or decrease in the rate of cooling is shown directly by the relative decrease or increase in the surface temperature excess (see Church, 1959). The second was the rather small amount of cooling by evaporation. The third, as we shall see later, was the small effect which the circulation of the blood has on the transfer of heat inside the insect.

## METHODS AND APPARATUS

Since so little of the heat generated in flight is lost by evaporation, it was not necessary either to use living insects in most of these experiments, or to control the atmosphere. Better results were obtained more easily with freshly killed insects in a regulated stream of air. They were heated internally with an electric current, and the heat generated was readily controlled. It was necessary only to have the room temperature reasonably steady and the air free from draughts, because conduction, radiation, and convection are little affected by the exact temperature of the air. Evaporative cooling could be ignored, particularly if the insects' spiracles were sealed.

The apparatus consisted of an open-jet wind tunnel, which blew a smooth, controlled stream over a mounted insect. The wind speed was measured with a hot-wire anemometer and regulated by a variable transformer connected to the

fan motor. The insect was heated by an alternating current produced by a high-frequency generator and its temperature excess was measured with a thermocouple. The specimen was supported in the wind by the heating electrodes and the thermocouple, which were clamped to a stand (Pl. 3, fig. 1).

*The wind tunnel and anemometer*

The wind tunnel (Pl. 3, fig. 1) was similar to that used by Weis-Fogh and Jensen (Weis-Fogh, 1956). A 19 cm., 60 W. fan forced air into a gradually expanded settling chamber. Seven radial vanes prevented the rotation of the fan from inducing a circular flow. In the wide section a 3 mm. mesh wire-gauze screen and a 'honeycomb' with holes 2·5 cm. square and 10 cm. deep further straightened out the flow and reduced turbulence. The sharp constriction leading to the 10 cm. wide throat or nozzle multiplied the speed of flow and further decreased turbulence and smoothed out irregularities in the velocity distribution across the jet (see Pankhurst & Holder, 1952).

Winds of from practically zero velocity to over 800 cm./sec. were available. Near the nozzle the air jet was the same speed across nearly its full width. At the highest speed slight turbulence was detected with very fine tufts of cotton, but none was apparent over the rest of the range. Large specimens and their mountings put into the jet hardly altered the rate of flow.

The wind velocity was measured with a constant-resistance hot-wire anemometer. The hot wire was 17 mm. of 25 $\mu$ platinum welded at each end to a thicker piece of platinum wire and was heated to about 600° C. The reference resistor was 26 B.S. gauge constantan. The anemometer was calibrated by attaching it to a revolving arm and moving it at different speeds through the air inside a tyre-shaped shield.

This type of instrument is very sensitive at low wind speeds (see Ower, 1949); changes in velocity could be detected ranging from considerably less than 1 cm./sec. in very slow winds to less than 5 cm./sec. in the fastest winds used. Ordinarily such changes in wind speed altered the temperature excess of a specimen by much less than 1 %. The absolute errors in measuring wind velocity caused by changes in air temperature and density were generally less than 3 % and usually no corrections were necessary.

*The heating apparatus*

The insects were heated internally by the current from a 10 kcyc. generator. An electronic voltmeter was used to measure the potential drop across the insect and, alternately, across a 1000 Ω resistor interposed between the insect and the generator, the method used by Krogh (1948). The current through the 1000 Ω resistor (and consequently through the insect also) was readily determined from the voltage and resistance. The energy converted to heat in the specimen at any moment was simply calculated from the current and the voltage across the insect. The maximum errors in calculating the large rates of heat input used with the largest insects were small: ± 1–2 % for the locusts, large dragonflies, and the largest bumble-bees and moths. In the small bumble-bees the possible error increased to about ± 4 %, and in the smallest moths to ± 6–7 %.

The heating electrodes used with most of the bees and moths were pieces of 0·32 mm. (30 B.S. gauge) platinum wire long enough to reach nearly across the insects' pterothorax. To one end of each electrode was soldered a 42-gauge platinum lead about 4 cm. long, which in turn was soldered to a sturdier length of copper. The lead was insulated by a glass tube (outside diameter 0·6 mm.) cemented to the base of the electrode and enclosing the fine platinum wire and the distal end of the copper. One electrode was inserted near the front of the pterothorax and the other near the back, and they were sealed in place with wax. The electrodes were completely inside the insect with only the insulated leads protruding. The insulators cut the heat loss appreciably, even from such fine wires, and in addition made convenient holders. The resistance of the wires was negligible compared with that of the insect. With some of the larger insects larger wire electrodes were used, and a pair of flat plates 3 mm. wide were used with the locusts.

*The thermocouples*

The temperature excess of the insect was measured with a 40-gauge copper-constantan thermocouple with the reference junction exposed to the air jet from the wind tunnel. The other junction was pushed into the centre of the pterothorax or just beyond it. (It was generally inserted at least 4 mm., but in the smallest insects the depth was necessarily less than this.)

The wires were thermally insulated 4 or 5 cm. back from the insect by a single glass tube. Combined with the cotton thread with which the copper wire was already wrapped this was a more effective and less cumbersome insulator than the two tubes used in earlier experiments (Church, 1959).

The heat loss from a thermocouple plus a pair of insulated electrodes was about the same as that from the type of thermocouple used on the locust flight mill (Church, 1959), perhaps less. The fraction of the heat generated in the insects which was lost through the electrodes and thermocouple was very small in the largest insects but it was substantial in the small ones. It was estimated to be between 1 and 5 % of the heat input in the models, locusts, and the large moths (denuded), bumble bees, and dragonflies; between 5 and 10 % in most of the noctuids and the smaller bumble bees and dragonflies; and perhaps 10–15 % in the small *Agrotis*.

*Mounting the insects and making the measurements*

The exact positions of the electrodes and thermojunction depended on the insect's shape. Generally they were placed among the legs so that they would not disturb the air flow over the body more than necessary. In *Schistocerca* the electrodes were inserted through the ventral surface of the pterothorax, with the thermojunction between them. In dragonflies also they were put in along the ventral mid-line, the thermojunction just behind the metathoracic legs, one electrode between the mesothoracic legs, and the other in the poststernum of the metathorax. In *Bombus* and in *Triphaena* and the other moths the thermojunction was inserted ventrally, but the electrodes were inserted in the side of the pterothorax.

The wings were removed from the insects and the stubs sealed with wax.

Supported by the electrode and thermojunction insulators, the insects were mounted rigidly, facing the air stream from the wind tunnel in approximately their natural attitudes (Pl. 2, fig. 2).

When the wind and heat had been turned on and adjusted to the appropriate levels 15 or 20 min. were allowed for the apparatus to warm up and for temperature equilibration. Four or five sets of readings a few minutes apart were then taken and averaged. Fluctuations in the temperature excess were small, generally less than $\pm 0{\cdot}1^{\circ}$ C.

*The size of the insect and the rate of heating*

There is considerable experimental and theoretical evidence that an animal's basal metabolism and the maximum power that the animal can produce are generally proportional to about the square (or a little more) of a representative linear dimension of the animal. However, the power required by an insect in flight to stay in the air must be proportional at least to its weight, or the linear dimension cubed (Hocking, 1953; Sotavalta, 1954). Accordingly, comparable series of insects in my experiments were heated in proportion to the volume of the pterothorax (except those referred to in Tables 1 and 2).

The volume of the pterothorax was estimated from the average of its diameters in three directions. In the locusts the first abdominal sternum was included in the pterothoracic capsule, and in the bumble-bees the pronotum (but not the rest of the prothorax) and, of course, the propodeum were included. Some of the measurements were rather arbitrary, particularly the length of the capsule in *Schistocerca* and the height in *Triphaena* and other moths. In the moths the tendency toward the incorporation of the coxae into the body made it difficult to establish an appropriate height for the capsule. But the estimated volumes are probably good enough for the comparisons that were made. Those of the well-rounded dragonflies and bumble-bees should be the most reliable. In *Schistocerca* the estimated volumes may have been up to 20% too small because of the cuboidal shape of the pterothorax.

The dragonflies in the experiments on heat conduction in the body (Table 3) were given about the same number of cal./mm.$^3$ as *Schistocerca* generates in flight—2·00 cal./min. for a 700 mm.$^3$ pterothoracic capsule (av. diam. 11 mm.). In reality, however, dragonflies probably produce heat considerably faster than this. Despite their large subcutaneous air sacs the thorax seems about as well filled with flight muscles as a locust's; less space in the centre is occupied by other air sacs and the gut. Besides, dragonflies are extremely strong fliers and their muscles may well work considerably harder.

The moths and bumble-bees in the convection experiments (Tables 4 and 5) were given $1\frac{3}{4}$ times as much heat per unit volume—2·67 cal./min. for a 525 mm.$^3$ pterothorax (av. diam. 10 mm.). This was the rate necessary in *Bombus lapidarius* and *Triphaena pronuba* to produce the temperature excesses that their live performance indicated would be about normal during steady flight. This rate is a little lower than various published rates of flight metabolism. Strong fliers among the Lepidoptera and Hymenoptera generally have been reported to produce two or three times as much energy per gramme body weight as *Schistocerca* (Weis-Fogh, 1952;

Hocking, 1953). Not only do these insects have relatively more flight muscle (Magnan, 1934; Weis-Fogh, 1952; Hocking, 1953) but their metabolic rates per gramme of muscle are also higher (Weis-Fogh, 1952).

### *The wind speed*

The wind speed was kept constant throughout each experiment, except where the effects of different speeds themselves were being measured. It was set, usually, at 250 or 300 cm./sec. (= 5·6 and 6·7 miles/hr.). These speeds were taken to be fairly close to the natural cruising speeds of strong, medium or large insects (Magnan, 1934; Hocking, 1953; Wigglesworth, 1953). If this estimate is rather conservative it is in keeping with the assumed metabolic rates. Many species are faster, especially the largest dragonflies and hawk moths, which go 5–10 m./sec. and sometimes reach 15 (Magnan, 1934; Wigglesworth, 1953). Furthermore, insects that ordinarily fly 2 or 3 m./sec. are capable of spurts twice as fast and would then lose more of their heat by convection.

In general, the temperature excesses given in the following sections should be reasonably realistic for the sort of insects used in the experiments, though not necessarily representative of particular species.

## CONDUCTION OF HEAT FROM THE FLIGHT MUSCLES TO THE SURFACE OF THE PTEROTHORAX AND TO OTHER PARTS OF THE BODY

In dead specimens heat exchange within the body is by conduction, but in living insects conduction is supplemented by convection—the circulation of the blood caused by the heart and other pulsatile organs and by the contractions of the flight muscles. Except in the dorsal blood vessel, circulation is sluggish, but it seemed possible that the flow of heat, expecially to the head and abdomen, might be appreciably increased.

The heat flow in the body was inferred from the internal temperature distribution. The effect of convection was determined by comparing the temperature gradients in dead and living animals. The insulating effects of air sacs were readily shown by filling them with water.

### *Temperature gradients in living and in artificially heated dead specimens*

Temperature excesses were measured at a number of representative points in freshly killed, artificially heated insects. The leads of the thermocouple were insulated with a thin flexible tube. The warm junction was always inserted 4 mm. into the specimen; when used to measure the temperature just beneath the surface it was run along under the integument. A second thermocouple with its junction among the flight muscles provided a reference temperature. Representative temperature excesses from fairly large male *Schistocerca* in a 280 cm./sec. wind are given in Table 1 (first column of figures). Similar measurements in *Bombus* and *Triphaena* are shown in Table 2. The temperature at each location was measured in at least four specimens.

Table 1. *Temperature excess (in °C.) distribution in male* Schistocerca gregaria

| | | Artificially heated in a 280 cm./sec. wind | Flying 270–300 cm./sec. |
|---|---|---|---|
| Pterothorax: | Centre (reference point) | 6·2 | 6·2 |
| | Top, beneath cuticle* | 5·8 (5·6)† | — |
| | Bottom, beneath cuticle* | 5·6 (5·4)† | 5·8 |
| | Side, beneath cuticle‡ | 5·2 (4·6)† | — |
| Prothorax: | Centre | 2·4 | — |
| Head: | Centre | 0·5 | — |
| Abdomen: | Centre of segment 2 | 2·8 | 3·0 |
| | Beneath cuticle of segment 2 | 1·6 (1·5)† | — |
| | Between segments 4 and 5 | 0·7 | 0·5 |
| | Beneath cuticle between segments 4 and 5 | 0·6 (0·6)† | 0·5 |
| | Centre of segment 8 | 0·3 | — |
| | Beneath cuticle of segment 8 | 0·2 (0·2)† | — |

* Measurements made where there were no superficial air sacs or extremely shallow ones.
† Estimated surface temperature excess.
‡ Thermojunction in superficial air sac of average thickness.

Table 2. *Temperature excess (in °C.) distribution in* Bombus *and* Triphaena

| | | Artificially heated in a 250 cm./sec. wind | Flying |
|---|---|---|---|
| *Bombus lapidarius*, large queens | | | |
| Pterothorax: | Centre (reference point) | 12·8 | 12·8 |
| | Beneath cuticle | 11·9 | — |
| | Surface of cuticle (estimated) | 11·5 | — |
| Abdomen: | Centre | 1·2 | 0·6 |
| *Triphaena pronuba*, medium or large males, partly rubbed | | | |
| Pterothorax: | Centre (reference point) | 7·9 | 7·9 |
| Abdomen: | Centre of segment 2 | 1·0 | 0·9 |
| | Centre of segment 5 | 0·4 | 0·5 |

Comparable measurements were made on live animals flying on a mill or in the wind tunnel (Tables 1 and 2, last columns). The temperatures were adjusted to a standard rate of heat production.

Rather unexpectedly there was no real difference between the living and dead specimens. In *Schistocerca* the temperature of the abdomen was more affected by being bent partly crosswise to the wind than by the circulation of the blood. In *Bombus*, rather oddly, the temperature of the abdomen was usually lower in the live bees. Internal convection therefore must play only a small part in the dissipation of heat from the flight muscles. The heat that passes from the pterothorax to the extremities must do so mainly by conduction even in active, living insects.

*Conduction from the pterothorax to the abdomen and other parts*

The temperature excess in the pterothorax was high right out to the surface. However, the temperature dropped off rapidly in the prothorax and abdomen (Tables 1, 2). The axial temperature gradient at the base of the abdomen in *Schistocerca*

was about 0·8° C./mm. and in the prothorax, next to its attachment to the pterothorax, it was about 0·9° C./mm. when the temperature excess of the centre of the pterothorax was 6·2° C. The cross-sectional areas of the attachment of the abdomen and the prothorax in a fairly large male are each 0·3–0·4 cm.$^2$. About half the area in each case is occupied by large air sacs, which are effective insulators. The coefficient of conductivity for water is 0·0014 cal./sec. cm. ° C., so if the heat that passes from the pterothorax through these regions does so mostly by conduction in a watery medium, the heat loss to the prothorax and abdomen together will be about 0·25 cal./min. In a locust generating about 2 cal./min. this is only 10–15 % of the heat produced. It is less than what must be dissipated from an equal area of the exposed surface of the pterothorax. Moreover, if so little heat flows to the prothorax and abdomen, it must follow that the wings and legs can be only the poorest of 'cooling fins'.

In most bees, moths, dragonflies, etc., probably even smaller portions of the heat from the flight muscles are conducted to the prothorax and abdomen. The attachments of the abdomen and prothorax are much narrower. In most moths examined less than half as much of the total surface of the pterothoracic capsule was occupied by these attachments as in *Schistocerca*. In dragonflies and bumble-bees it was still less. Moreover, the broadest of the attachments were interrupted by air sacs as in the locusts, especially in the dragonflies and noctuid moths.

However, in an insect whose thorax is well insulated by air sacs or hair the relative amount of heat conducted from the pterothorax to the rest of the body will be somewhat larger than in an uninsulated insect, because the temperature gradients between the thorax and the other parts will be steeper. Similarly, a stationary insect exercising its muscles in a place sheltered from the wind will lose a greater portion of its heat indirectly through the prothorax, head, and abdomen, etc., than will a flying insect or one exposed to a strong wind.

### *Conduction to the surface of the pterothorax*

The temperature excess decreases by only a small fraction from the centre to the surface of the pterothoracic capsule itself. There are two reasons for this. First, the flight muscles are not concentrated in the centre of the pterothorax but in the outer part, roughly in the form of a thick-walled, hollow sphere. Secondly, the temperature curve along the diameter of the capsule would be fairly flat even if the flight muscles did fill the pterothorax, because the heat is produced throughout the muscles rather than at one point. This is illustrated by some temperature measurements made in an 8 mm. diameter, thin-walled cylindrical model mounted across a 240 cm./sec. wind. The model was filled with saline and heated with the high-frequency current. When the temperature excess in the centre of the model was 10·1° C., it was 10·0° C. 2 mm. in from the surface, half way between the surface and the axis. It decreased faster toward the outside, but even at the surface it averaged about 9·2° C., hardly 10 % less than at the centre.

In *Bombus* the temperature excess at the surface of the pterothorax was only about 10 % lower than at the centre (Table 2). Even in *Schistocerca*, which has

an extensive, though generally rather thin, layer of air sacs between the muscles and the integument, the surface temperature excess was only about 20% lower (Table 1). Thus the surface temperature of the pterothorax can usually be derived directly from the internal temperature. This is not so, however, in insects such as dragonflies, in which subcutaneous air sacs are extremely well developed.

### *Insulation of the pterothorax by superficial air sacs*

In dragonflies air sacs form an almost continuous, deep air space between the flight muscles and the skin, lining nearly the entire pterothorax except for the ventral muscle attachments. Most of the dorsal surface can be insulated as well as the pleura because of the very narrow attachments of the flight muscles to the wing bases. The air sacs were measured in a number of dragonflies of the same size as those used for the temperature measurements below. In the large dragonflies *Aeschna* and *Anax* about 80% of the exposed surface of the pterothoracic capsule was insulated. The maximum thickness of the superficial air sacs was about 1·3 mm. and the average was between 0·8 and 0·9 mm. In the smaller species *Sympetrum striolatum* air sacs underlay nearly 85% of the surface. Their maximum thickness was 0·6 mm. and their average 0·3–0·4 mm.

The insulating effects of the air sacs were demonstrated with artificially heated, freshly killed animals. The mouth and anus of each insect were sealed and several weak spots in the integument reinforced with wax. Readings of the equilibrium temperature excess of the insect were taken in a 250 cm./sec. wind. Then the insect, mounted on the thermocouple-electrodes assembly, was submerged in a dish of water containing a few drops of detergent in a vacuum chamber and the air sacs were filled with water. The insect was wiped off and its spiracles were sealed; then it was dried in a wind until absolutely no moisture could be seen anywhere on the surface with a dissecting microscope. The insect was replaced exactly in its former position in the wind tunnel jet, and temperature readings were taken as before. The results are shown in Table 3.

Under these conditions the air sacs were responsible for increases of 4–5° C. in

Table 3. *Effect of insulation by air sacs on the temperature excess of male dragonflies in a 250 cm./sec. wind*

| | Average diameter of ptero-thorax (mm.) | Heat input (cal. min.) | Temperature excess (° C.) | | | Difference in temperature, percentage of temperature excess with water in air sacs |
|---|---|---|---|---|---|---|
| | | | With air in air sacs | With water in air sacs | Differ-ence | |
| *Aeschna cyanea* Müll. | 11 | 1·94 | 13·0 | 8·3 | 4·7 | 57 |
| *Anax imperator* Leach | 10·7 | 1·78 | 11·8 | 7·7 | 4·1 | 53 |
| *A. cyanea* | 10·3 | 1·63 | 12·6 | 7·5 | 5·1 | 68 |
| *Aeschna grandis* Linn. | 9·7 | 1·34 | 10·1 | 6·4 | 3·7 | 58 |
| *Sympetrum striolatum* Charp. | 6·5 | 0·40 | 4·7 | 3·8 | 0·9 | 24 |
| *S. striolatum* | 6·5 | 0·40 | 4·5 | 3·3 | 1·2 | 36 |
| *S. striolatum* | 6·5 | 0·40 | 5·1 | 4·0 | 1·1 | 27 |
| *S. striolatum* | 6·5 | 0·40 | 4·9 | 4·0 | 0·9 | 23 |

the temperature excesses of *Aeschna* and *Anax* and increases of about 1° C. even in *Sympetrum*. The increases in the large species averaged nearly 60 % of the temperature excesses of the uninsulated insects.

As a check on the results in Table 3 a simple calculation was made to see whether the results from the large and small insects were consistent. If the heat produced in a body has to escape by conduction through an insulating layer a certain temperature gradient across the layer is required. The temperature gradient must be directly proportional to the amount of heat escaping and inversely proportional to the area of the insulation. In addition, the total temperature drop across the layer must be directly proportional to its thickness. The average linear dimensions of the pterothoracic capsules in the large dragonflies and in *Sympetrum*, respectively, were 10–11 and 6–7 mm., and the average thicknesses of the insulating layers were 0·8–0·9 mm. and 0·3–0·4 mm. The area of insulation was roughly proportional to the square of the average linear dimension of the pterothorax and the heat produced proportional to the cube. Therefore, one would expect that the average temperature drop across the air-sac layer in *Aeschna* and *Anax* would be very nearly four times that in *Sympetrum*. In the experiments this drop was represented by the fall in temperature caused by filling the air sacs with water. (Actually, the temperature drop must have been a little larger than the decrease produced experimentally because the entire surface of the pterothorax was not insulated.) As this averaged 4·4° C. in *Aeschna* and *Anax* and 1·0° C. in *Sympetrum* the relationship was close to the one expected.

Experiments with some model insects—waxed paper cylinders of appropriate sizes filled with saline—also supported the results given above. Air jackets like those of the large dragonflies produced effects almost exactly equivalent to the effects recorded in the insects.

In a live dragonfly internal convection must reduce the air sacs' insulating value to some extent, but probably only slightly. If the rate of thoracic ventilation is comparable to that in flying *Schistocerca*—350 cm.$^3$/g. of insect/hr. (Weis-Fogh, 1953)—the average air speed through the subcutaneous air sacs would be less than 1 cm./sec., even if all the air entered the first pair of spiracles and went through the subcutaneous air sacs. This rate of flow is very small compared with the speed of the wind outside the insect.

## HEAT LOSS BY LONG-WAVE RADIATION

The relative effect of a certain amount of radiation on an insect's temperature excess depends, of course, on the metabolic rate, and the absolute effect depends on the rate of cooling by convection and what little evaporation there is. If the radiation can be calculated accurately enough by the Stefan–Boltzmann formula, and if the metabolic rate is known, then the relative effect of the radiation is predictable. Although it seemed that reasonable estimates could now be made of the values needed for the calculations, the results could not be defended with confidence. But evidence of the reliability of such calculations could be obtained by measuring

the actual effects of radiation from the insect on its temperature in a constant air stream. Accordingly, temperatures were measured in *Schistocerca* alternatively permitted to radiate heat to the environment and prevented from doing so.

### *Special methods and apparatus*

The insects were mounted as before. A radiator warmed to the same temperature as the surface of the experimental insect's pterothorax, in which the animal could be almost entirely enclosed, was used to prevent heat loss by radiation. The radiant heat loss was derived from the 'metabolic rate' and the temperature excess of the surface of the pterothorax, shielded and exposed. The changes in the temperature excess themselves showed the practical effects of long-wave radiation on the temperature of the flight muscles. Measurements were made both in the laboratory and outside to obtain a measure of radiant cooling indoors, where the other experiments were done, and a realistic idea of its effect under natural conditions.

The radiator was a metal cylinder, 18 cm. in diameter, blackened with smoke inside and covered by a water-jacket. A specimen was mounted so that the cylinder could be slid over it without disturbing it or interfering with the jet from the wind tunnel. A correction had to be made for the radiation that escaped through the open ends of the cylinder; the radiator blocked only 90 % of the solid angle around the insect. The 'air' junction of the thermocouple was encased in a small, polished bead of solder so that it would stay at air temperature and not be affected by radiation. The theoretical estimates of the amount of radiation were subject to several qualifications. It was most practical to regard all the radiation as coming from the surface and to use the surface temperature of the pterothorax as the temperature governing the rate of emission; however, this is not strictly correct because insects are partly transparent to long-wave radiation. The emissivity could not be known precisely, but according to Digby (1955) 0·75 is about right for *Schistocerca*. Neither could the area of the radiating surface of the pterothoracic capsule be determined exactly, but it could be measured approximately. In the laboratory the average surface temperature of the surroundings could be obtained fairly accurately, but outdoors this was more difficult.

### *Radiation from an insect in the laboratory*

A representative set of results obtained in the laboratory with a male locust is tabulated below. The insect weighed close to 2 g. and the wind speed was 300 cm./sec.

| | |
|---|---|
| Air temperature | 19·8° C. (range ±0·1°) |
| Surface temperature of radiator | 26·3° C. (range ±0·5°) |
| Temperature of room walls | 19·1° C. = 292·3° K. (range ±0·5°) |
| Heat input | 1·95 cal./min. |

| | Exposed | Shielded |
|---|---|---|
| Temperature excess of insect (centre of pterothorax) | 7·2° C. (range ±0·1°) | 7·7° C. (range ±0·2°) |
| Estimated temperature excess of surface of pterothorax | 5·8° C. | 6·3° C. |
| Estimated temperature of surface of pterothorax | 25·6° C. = 298·8° K. | 26·1° C. |
| Drop in temperature excess caused by long-wave radiation | About 0·5° C. | |

The rate of heat loss by convection was proportional to the surface temperature excess. Therefore, of the heat dissipated from the surface of the pterothorax, the fraction lost by radiation must have been about 0·5/6·3, the temperature drop divided by the temperature excess of the surface when radiant loss was prevented. In a previous section about one-eighth of the heat generated in the insect was calculated to be conducted from the pterothorax to other parts of the body, leaving seven-eighths to escape directly from the surface of the pterothorax. If allowance is made for the shield being only 90% effective, then about

$$0{\cdot}5/6{\cdot}3 \times \tfrac{7}{8} \times \tfrac{10}{9} \times 1{\cdot}95 = 0{\cdot}15 \text{ cal./min.}$$

must have been radiated from the pterothorax. Some heat was also radiated from other body segments but this was included in the fraction conducted from the pterothorax to the other parts.

How does that value compare with a theoretical estimate?

$$H = 8{\cdot}22 \times 10^{-11} \times E(T_1^4 - T_2^4),$$

and if we take the emissivity of the locust to be 0·75 and substitute the pterothoracic surface temperature (exposed) and the wall temperature for $T_1$ and $T_2$, $H = 0{\cdot}042$ cal./min. cm.$^2$. For a 4 cm.$^2$ surface this would be 0·17 cal./min. If the pterothoracic capsule of a 2 g. male locust, maximum width 10 mm., is cut up and laid flat, the exposed surface comes to 4 or 4·1 cm.$^2$, disregarding the finer convolutions.

The agreement between the experimental and theoretical results was usually about as close as in this example. In most cases, as in this one, the temperature decrease produced by radiation seemed to be a little less than theory and the assumed values suggested. Allowing for error, it is safe to say that it was not greater and that in the laboratory less than 10% of the 'metabolic heat' was dissipated from the pterothorax by radiation.

In the laboratory radiation can conveniently be considered proportional to the temperature excess. Within our restricted range $T_1^4 - T_2^4$ is approximately proportional to $T_1 - T_2$, and the air temperature usually can be substituted for $T_2$ instead of the wall temperature. Because the amount of radiation from an insect is relatively small the error involved in this approximation is unimportant. We have seen (Church, 1959) that at room temperature in the laboratory an insect's temperature excess is almost proportional to the heat generated—or the rate of heat loss is proportional to the temperature excess. If cooling by radiation, as well as by convection, is proportional to the temperature excess of the surface, that is indeed what one would expect.

In the laboratory a small insect would lose even less of its heat by radiation than a large one if the rest of it is dissipated mainly by convection. The large insect's temperature excess would likely be higher than the small one's but both radiation and convection are proportional to the temperature excess. However, the larger insect has more surface, and radiation is proportional to the surface area whereas convective cooling is not (see the next section).

*Radiation from an insect to the sky*

The apparatus was moved out on to the roof of the laboratory and measurements were made on several clear, still summer nights. The following results, obtained with a male locust weighing just over 2 g. in a 320 cm./sec. wind, were typical.

| | |
|---|---|
| Air temperature | 13·5° C. (range ±0·3°) |
| Surface temperature of radiator | 19·2° C. (range ±0·4°) |
| Temperature of roof | 18·0° C. (range ±0·2°) |
| Heat input | 2·17 cal./min. |

| | Exposed | Shielded |
|---|---|---|
| Temperature excess of insect (centre of pterothorax) | 6·6° C. | 7·0–7·1° C. |
| Estimated temperature excess of surface of pterothorax | 5·3° C. | 5·7–5·8° C. |
| Estimated temperature of surface of pterothorax | 18·8° C. = 292·0° K. | 19·2–19·3° C. |
| Drop in temperature excess caused by long-wave radiation | 0·4–0·5° C. | |

The 0·4–0·5° decrease in the temperature excess whenever the shield was removed indicated that the heat loss by radiation from the pterothorax was between 0·15 and 0·18 cal./min., less than 10% of the heat generated in the insect.

It might have been expected to be a little greater. The roof was nearly the same temperature as the locust so the net loss from the lower half of the insect was extremely small, perhaps 0·01 cal./min. The effective temperature of the clear sky, in this context, is said to be about 0° C. (Parry, 1951). The radiation from the upper hemisphere of the pterothorax to the sky should then have been about

$$2 \times 8{\cdot}22 \times 0{\cdot}75 \times 10^{-11} (292{\cdot}0^4 - 273{\cdot}2^4) = 0{\cdot}21 \text{ cal./min.}$$

Other specimens on other clear nights gave similar results. One need only assume that the effective temperature of the sky was actually a few degrees above zero to get the experimental and theoretical figures to coincide. Though the stars were visible and the sky seemed clear it may not have been perfectly so. When it was cloudy radiant cooling was noticeably decreased.

If the temperature had been higher there would have been more radiant cooling. But even if the insect had been 35–40° C. warmer than the clear sky instead of less than 20° C. warmer radiation still would have accounted for less than 20% of its metabolic heat. It would have reduced the insect's pterothoracic temperature by about 1° C.—just enough, perhaps, to be of some value to the insect if it were in danger of overheating.

A smaller insect would tend to lose a larger proportion of its heat by radiation to the sky (in contrast to the relationship in the laboratory) because of its larger surface-to-volume ratio and because the amount of radiation outdoors is not closely tied to the insect's temperature excess. But it should be remembered that many insects generate heat much faster for their size than *Schistocerca* does.

In general, radiation to a cold sky obviously must help to moderate an insect's temperature; the warmer the insect gets the more it is cooled by radiation. However, the effect is too small to be of practical importance.

The absorption of sunshine is influenced considerably by an insect's orientation and its visible colour, but the emission of long-wave radiation is not.

## HEAT LOSS BY CONVECTION

Of the four ways by which heat escapes from the pterothorax the three that have been considered do not account for the major part of the heat produced. Imagine an insect typical of those discussed here flying in a natural environment—the air perhaps 20° C. and moderately dry, and the sky mostly clear. Of the heat produced in the pterothorax the following fractions might be dissipated by the mechanisms considered so far: by evaporation, likely not much more than 5–10 %; by radiation, probably about 10–15 %; by conduction to other parts of the body, about 5–15 %. The total is 20–40 %, which means that about 60–80 % of the heat must be dissipated by convection.

The important factors in convection have already been mentioned. For convenience the effects of surface covering were measured first—how the 'fur' on the thorax of a bee or moth influences its temperature in winds of various speeds and how different coats compare in insulating value. The relationships between the temperature excess of naked specimens and their size and shape and the air speed were then shown, and their rates of cooling were compared with the rates of cooling of objects of regular shape.

### *Special methods*

The temperature excesses of artificially heated furry insects mounted in their natural attitudes facing the wind from the tunnel were compared directly with the insects' temperature excesses after all the hair had been removed from the thorax, the back of the head, and the base of the abdomen. The tarsi and wings were removed and the cut ends sealed before the experiments.

In the moths the hair and the scales were easily rubbed off with a pipe cleaner, but the hair had to be stripped from the bumble-bees with paraffin wax. Care was taken not to break the integument and whenever such injury might have occurred the site was lightly waxed. (A thin layer of wax, even when applied to the whole thorax, had a barely noticeable effect on the rate of convective cooling.) The hairy legs were cut off and the ends sealed.

Since the experiments were done indoors with dead specimens, conditions were unnatural but not seriously so. The air in the laboratory was over 75 % saturated and the insects were not breathing, so evaporation did not take more than 2 or 3 % of a specimen's heat. Radiation accounted for something less than the 10–15 % of the metabolic heat radiated outdoors—probably about half that much in a denuded or poorly insulated insect. However, the 5–10 % average loss (in a naked insect) through the thermojunction and electrode wires tended to compensate for the reductions in evaporation and radiation. In all, in naked specimens a little less heat was lost by other mechanisms than convection than would be in natural flight outside.

Well-insulated insects must have lost somewhat more heat than naked ones by routes other than convection. The effects of fur and the wind velocity on the proportion of the heat dissipated from the pterothorax by convection will be taken into consideration in the discussions that follow. Outdoors the effects are similar but less well marked.

### *Convective cooling and insulation by body hair and scales in bumble-bees and moths*

The experimental results are summarized in Tables 4 and 5. Let us consider the bumble-bees first.

Table 4. *Effects of insulation by body hair and of size on the temperature excess of bumble-bees in a 300 cm./sec. wind*

| | Average diameter of ptero-thoracic capsule (mm.) | Heat input (cal./min.) | Temperature excess (° C.) | | | Difference in temperature, percentage of temperature excess with hair removed |
|---|---|---|---|---|---|---|
| | | | With hair intact | With hair removed | Difference | |
| *Bombus terrestris* Linn. ♀♀. Thoracic coat fairly dense, somewhat recumbent, slightly tangled; average depth 1¼–1½ mm. | 8·8 | 1·84 | 11·3 | 6·8 | 4·5 | 66 |
| | 8·5 | 1·64 | 10·2 | 5·9 | 4·3 | 73 |
| | 8·3 | 1·54 | 9·1 | 6·1 | 3·0 | 49 |
| | 8·3 | 1·54 | 11·4 | 7·3 | 4·1 | 56 |
| | | | | Mean | 4·0 | |
| *B. lapidarius* Linn. ♀♀. Coat dense and fine, erect; 1–1½ mm. | 8·3 | 1·54 | 10·8 | 6·9 | 3·9 | 57 |
| | 8·3 | 1·54 | 12·2 | 7·2 | 5·0 | 69 |
| | 8·3 | 1·54 | 11·1 | 6·5 | 4·6 | 71 |
| | 8·3 | 1·54 | 10·6 | 6·8 | 3·8 | 56 |
| | 8 | 1·37 | 9·2 | 5·6 | 3·6 | 64 |
| | | | | Mean | 4·2 | |
| *Psithyrus vestalis* Foucr. ♀♀. Coat relatively short, very thin, erect; about 1 mm. *P. rupestris* Fabr. ♀* | 8·5 | 1·64 | 8·5 | 6·0 | 2·5 | 42 |
| | 8·5 | 1·64 | 11·2 | 7·7 | 3·5 | 45 |
| | 8·5 | 1·64 | 8·9 | 6·6 | 2·3 | 35 |
| | 8·3 | 1·54 | 10·2 | 7·1 | 3·1 | 44 |
| | | | | Mean | 2·9 | |
| *Bombus lapidarius* ♀♀. Coat dense and fine, erect; about ¾ mm. | 5·8 | 0·53 | 7·2 | 4·3 | 2·9 | 67 |
| | 5·5 | 0·45 | 6·5 | 3·7 | 2·8 | 76 |
| | 5·3 | 0·40 | 7·2 | 4·4 | 2·8 | 64 |
| | 5·3 | 0·40 | 6·9 | 4·0 | 2·9 | 73 |
| | | | | Mean | 2·9 | |
| *B. derhamellus* Kirby ♀♀. Coat somewhat thin and uneven, erect; ¾–1 mm. | 5·3 | 0·40 | 6·3 | 4·0 | 2·3 | 58 |
| | 5·3 | 0·40 | 5·6 | 3·7 | 1·9 | 51 |
| | | | | Mean | 2·1 | |
| *B. terrestris* ♂♂. Coat long, somewhat thin; 1¼–1⅝ mm. | 5·8 | 0·53 | 5·5 | 3·5 | 2·0 | 57 |
| | 5·3 | 0·40 | 5·8 | 3·8 | 2·0 | 53 |
| | 5·3 | 0·40 | 7·3 | 4·2 | 3·1 | 74 |
| | | | | Mean | 2·4 | |
| *B. lapidarius* ♂♂. Coat fairly long, thin, erect; 1–1¼ mm. | 5·3 | 0·40 | 3·9 | 2·6 | 1·3 | 50 |
| | 5·3 | 0·40 | 5·6 | 3·4 | 2·2 | 65 |
| | 5·3 | 0·40 | 5·1 | 3·7 | 1·4 | 38 |
| | 4·5 | 0·24 | 4·9 | 3·0 | 1·9 | 63 |
| | 4·5 | 0·24 | 5·3 | 3·4 | 1·9 | 56 |
| | | | | Mean | 1·7 | |

* Description of coat same as *P. vestalis*.

Table 5. *Effects of insulation by hair and scales and of size on the temperature excess of moths in a 300 cm./sec. wind*

| | Average diameter of pterothorax (mm.) | Heat input (cal./min.) | Temperature excess (° C.) With hair intact | Temperature excess (° C.) With hair removed | Temperature excess (° C.) Difference | Difference in temperature, percentage of temperature excess with hair removed | Ratio of temperature difference to average pterothoracic diameter |
|---|---|---|---|---|---|---|---|
| *Sphinx ligustri* Linn. ♂♂. Coat very dense, fine, somewhat tangled; average depth 1½–2½ mm. | 9 | 1·94 | 18·2 | 9·3 | 8·9 | 96 | 0·99 |
| | 9 | 1·94 | 15·7 | 7·5 | 8·2 | 109 | 0·91 |
| | 8·5 | 1·63 | 17·4 | 8·0 | 9·4 | 118 | 1·11 |
| | | | | Mean | 8·8 | | 1·00 |
| *Laothoe populi* Linn. ♂♂. Coat very dense and fine, tangled; 2–2½ mm. | 7·3 | 1·05 | 16·1 | 7·7 | 8·4 | 109 | 1·15 |
| | 7 | 0·90 | 13·1 | 5·4 | 7·7 | 143 | 1·10 |
| | | | | Mean | 8·1 | | 1·13 |
| *Triphaena pronuba* Kubb. Coat not uniformly dense, recumbent; about 1 mm., less in centre of dorsum ♂ | 6·8 | 0·84 | 8·7 | 5·3 | 3·4 | 64 | 0·50 |
| ♂ | 6·3 | 0·67 | 6·3 | 4·0 | 2·3 | 58 | 0·36 |
| ♀ | 6·3 | 0·67 | 8·2 | 4·5 | 3·7 | 82 | 0·58 |
| ♀ | 6·3 | 0·67 | 9·4 | 5·7 | 3·7 | 65 | 0·58 |
| | | | | Mean | 3·3 | | 0·51 |
| *Thalpophila matura* Huf. ♂♂. Coat dense, moderately fine, partly recumbent; 1–1½ mm. | 4·7 | 0·27 | 5·2 | 3·3 | 1·9 | 58 | 0·41 |
| | 4·7 | 0·27 | 5·6 | 3·4 | 2·2 | 65 | 0·47 |
| | 4·7 | 0·27 | 5·9 | 3·1 | 2·8 | 90 | 0·60 |
| | | | | Mean | 2·3 | | 0·49 |
| *Plusia gamma* Linn. Coat dense, fine, somewhat recumbent; 1½–2 mm. ♀ | 4·7 | 0·27 | 6·4 | 3·3 | 3·1 | 94 | 0·66 |
| ♂ | 4·5 | 0·24 | 4·9 | 3·0 | 1·9 | 63 | 0·42 |
| | | | | Mean | 2·5 | | 0·54 |
| *Malacosoma neustria* Linn. ♂. Coat dense, very fine, somewhat curly; 1½–2 mm. | 4·3 | 0·22 | 7·8 | 4·0 | 3·8 | 95 | 0·88 |
| *Agrotis cinerea* Hueb. ♀♀. Coat dense, fine, fairly erect; 1–1½ mm. | 3·7 | 0·13 | 3·9 | 2·3 | 1·6 | 70 | 0·44 |
| | 3·7 | 0·13 | 4·1 | 2·2 | 1·9 | 86 | 0·52 |
| | | | | Mean | 1·8 | | 0·48 |
| *Agrotis puta* (Hueb.) ♂♂. Coat dense, fine, fairly erect; 1–1¼ mm. | 3·2 | 0·08 | 3·2 | 1·5 | 1·7 | 113 | 0·54 |
| | 3·2 | 0·08 | 4·7 | 2·7 | 2·0 | 74 | 0·63 |
| | 3·2 | 0·08 | 4·5 | 2·4 | 2·1 | 88 | 0·66 |
| | | | | Mean | 1·9 | | 0·61 |

The bees' hair provided a well-marked and fairly consistent amount of insulation (Table 4). As an average in the three species of *Bombus* it increased the pterothoracic temperature excess by just over 60% in a 300 cm./sec. wind. The four *Psithyrus* were less well insulated. 15–20% of the insulation of the thorax was provided by the abundant long hair on the femora and tibiae. It is interesting that it was most effective when the legs were allowed to dangle loosely as they do in flight.

A closer examination of the results in Table 4 will be simpler and more useful if we consider the absolute rather than the relative temperature difference between the denuded and normal states. The absolute difference shows the efficiency of the insulation better, since it represents the original temperature drop across the insulating layer.

As expected, the hair on the smaller specimens generally did not cause so large an absolute temperature increase as it did in the larger ones. The temperature drop across a 'layer 'of fur of a certain quality and depth should be proportional to the diameter of the pterothoracic capsule if the heat generated in the insect is proportional to the volume of the capsule, and the average area of insulation through which the heat must escape is proportional to its surface. Nevertheless, even in the smaller bumble-bees the temperature increase was seldom much less than 2° C., which is sufficient, on occasion, to be important.

Comparison of the *Bombus terrestris* and *lapidarius* queens with the female *Psithyrus vestalis* and *rupestris*, which are about the same size, gives an idea of the effects of the density of the coat and the length of hair. The *Psithyrus* coats were somewhat shorter and much thinner than those of the *Bombus* queens and because of this they produced an average temperature increase of only 2·9° C. compared with the average increases in the *Bombus* of 4·0 and 4·2° C.

Comparison of the *B. lapidarius* and *derhamellus* workers with the *B. terrestris* and *lapidarius* males suggests that the density rather than the length of the hair is most important. The insects in the two groups were about the same size, if the two smallest *lapidarius* males are excluded. The males' coats were 1½ times as deep as the workers' but the males were no better insulated for it. The *lapidarius* males, indeed, were poorer than the *lapidarius* workers, despite their very hairy appearance; though their hair was longer it was not so dense. Notice also that the average temperature increase in the *B. lapidarius* workers was as large as it was in the female *Psithyrus*. The former had somewhat shorter coats and were considerably smaller; but their hair was fine and dense.

Other experiments on several large bumble-bees confirmed that the length of hair is not an especially effective factor. The bees' hair was removed in two stages: first, the legs were cut off and the body hair was clipped to about one-third its original length; secondly, it was removed entirely. The amputation and clipping caused an appreciable temperature drop, but never one as large as half the total drop effected by removing the hair completely.

The reasons are quite simple. If we imagine the insulation to be in several layers around the body the outer layers will have greater areas through which the heat can pass and therefore will be less effective barriers. Moreover, because each addi-

tional layer increases the area of the outer boundary of the insulation and the 'surface' from which the heat is finally removed by freely moving air, the 'surface' temperature need not be so high for all the heat to be dissipated. The over-all effect of extra depths of insulation thus will be further diminished. Finally, the hairs are less closely spaced at their tips, in the 'layers' farthest from the body, and likely offer less resistance to the flow of air.

The moths (Table 5) were considerably better insulated than the bumble-bees, which was to be predicted because their fur was obviously denser. The superb coats of the hawk moths, *Sphinx ligustri* and *Laothoe populi*, produced an average increase of 115 % in the temperature excess in a 300 cm./sec. wind. In absolute terms this generally meant an increase of 8 or 9° C. In the smaller moths, which were nearly all noctuids, the increase in temperature excess was generally from 60 to 90 %. The absolute increase tended to diminish, as expected, with the insects' size; yet even in the small *Agrotis* it was nearly 2° C.

As we have already remarked, the temperature increase produced by an insulating coat should be proportional to the diameter of the insulated body if other things are equal. Thus it is convenient to use the ratio of the temperature increase to the average diameter of the pterothorax to compare the insulating efficiency of the different species' coats. This expression for each of the moths is given in the last column of Table 5.

In general, the insects whose coats looked the best—the sphingids, particularly *Laothoe* with its exceptionally fine, dense, long hair, and *Malacosoma neustria*, whose tarsi, even, were profusely hairy—proved in fact to be the best insulated.

Clearly the differences in the length of hair among the noctuids, most of which had coats of about the same density, had small effects at most on the temperature increase per millimetre thoracic diameter. On the same basis the difference between the hawk moths and the noctuids was rather large; the temperature increase per unit diameter was twice as high in *Sphinx* and *Laothoe*, no doubt partly because their hair was $1\frac{1}{2}$ or 2 times as long but probably more because it was denser than the noctuids'. Similarly, in *Malacosoma* it was probably the density more than the length of the hair that was so effective.

### *Efficiency of the insulation provided by an insect's coat*

The relative efficiency of an insulating covering can be established by comparing it with a perfectly still layer of air through which heat is transferred only by conduction. Let us take as an example the first specimen of *Sphinx ligustri* in Table 5. The 'metabolic rate' was 1·94 cal./min. and the coat of hair and scales on the thorax raised its temperature by 8·9° C. About a third or more of the heat may have been dissipated by radiation and evaporation and by conduction to the thermojunction and electrode wires and the abdomen, etc., leaving 1·3 cal./min., or 0·022 cal./sec., to pass through the fur and be carried away by convection. If we take the pterothoracic capsule to be a sphere and its diameter to be 9 mm. the capsule's surface can be estimated at just over 2·5 cm.$^2$. The conductivity of air is $6{\cdot}2 \times 10^{-5}$ cal./cm.

sec. °C. Therefore, as a first approximation the depth of still air that would be required to produce an equivalent temperature increase would be

$$(6{\cdot}2 \times 10^{-5} \times 2{\cdot}5 \times 8{\cdot}9 \times 10)/0{\cdot}022 = 0{\cdot}6 \text{ mm.}$$

Two corrections, however, should be made to this figure: one because the average area of the air layer through which the heat is conducted would be slightly larger than the surface of the bare capsule, and another because the outer 'surface' where convection begins would also be larger than the surface of the bare capsule and the equilibrium temperature there would be reduced. The adjustments would raise the figure to about 0·9 mm. The actual depth of the fur was about 2 mm., just 2 or $2\frac{1}{2}$ times as great.

A sphinx moth's hair is evidently an effective barrier to convection. However, comparison of *Sphinx* with some well-insulated models emphasized just how effective its coat is. Indeed, it proved difficult to insulate a small body more efficiently than *Sphinx* is insulated, if the depth of the insulation is taken into account. (Most of the other moths and the bumble-bees, of course, would be easier to improve on.)

The models used for comparison were saline-filled waxed paper cylinders, 8 mm. in diameter and 35 mm. long thinly shellacked on the outside. Some were insulated with material applied to the surface while the shellac was still wet; the controls remained bare. The most effective insulation was a dense layer of cotton wool 4 mm. deep, over which was slipped a cylinder of thin paper, the ends of which were left open.

In the uninsulated models mounted across a 300 cm./sec. wind, a 4·0 cal./min. heat input produced an average temperature excess of 5·0° C. In the cotton-insulated models the average temperature excess rose to 19·1° C. As before, the depth of the layer of still air that would be necessary to produce the same rise in temperature was calculated.

About 70% of the heat supplied, or about 2·8 cal./min., had to go through the insulation. The rest escaped through the wires and from the bare ends of the models. The area of the insulated surface of each model was 8·8 cm.$^2$, but the average area of the still air layer through which the heat would have to pass would be somewhat larger. If the required air layer is anticipated to be nearly $2\frac{1}{2}$ mm. deep the area can be estimated as $11\frac{1}{2}$ cm.$^2$. If the temperature drop across the still air were 14·1° C. then the layer would have to be about

$$(6{\cdot}2 \times 10^{-5} \times 11{\cdot}5 \times 14{\cdot}1 \times 60 \times 10)/2{\cdot}8 = 2{\cdot}1 \text{ or } 2{\cdot}2 \text{ mm. deep.}$$

However, the temperature drop across the layer would have to be more than 14·1° C. because the outer surface, where convection would begin, would be larger and cooler than that of the bare model. Therefore, the estimate must be increased to about 2·3 mm. At any rate it would not come to much more than half the depth of the cotton.

The difference is the result of two factors. First, there was some air movement in the cotton, especially at the ends of the cylinder where it was not protected by the outer paper shield. Secondly, a certain amount of heat got across the layer of cotton by long-wave radiation.

These models were not strictly comparable to the insects because of their shape. Nevertheless, the fact that the cotton insulation fell considerably short of the still-air standard makes the hawk moths' fur seem all the better.

Similar models insulated with very coarse 'coats' made up of shellacked wooden spines suggested what sort of covering would just begin to reduce convective cooling. The spines were 4 mm. long and about $\frac{1}{2}$ mm. across and were spaced 20 or 30/cm.$^2$ The covering had only a small effect; in a 300 cm./sec. wind it generally increased the temperature excess by less than 5 %. By way of contrast, tufts of hair, 4 mm. long, applied perpendicular to the surface and spaced 2000–3000 hairs/cm.$^2$ caused an average increase of 95 % in the temperature excess.

Probably any hairy or spiny covering that occurs on an insect will decrease rather than increase the rate of cooling. But the effect of a few spines scattered over the body, as in many flies, scarcely needs be considered.

*Effects of body shape and size on convective cooling of naked insects*

The excess temperatures of the denuded bumble-bees and moths are plotted against the average diameters of their pterothoracic capsules in Text-fig. 1. The temperatures of the moths were most often a little higher than those of bees of the same size; however, the small differences easily might have been due to discrepancies in estimating the insects' size. The excess temperatures of the dragonflies in a 300 cm./sec. wind, with the same 'metabolic rate' and with their air sacs filled with water, are also shown in the figure. They were higher than either the bees' or moths', probably because the dragonflies still had their legs intact and some light hair on the thorax. The differences in body shape among the three orders apparently were not very important.

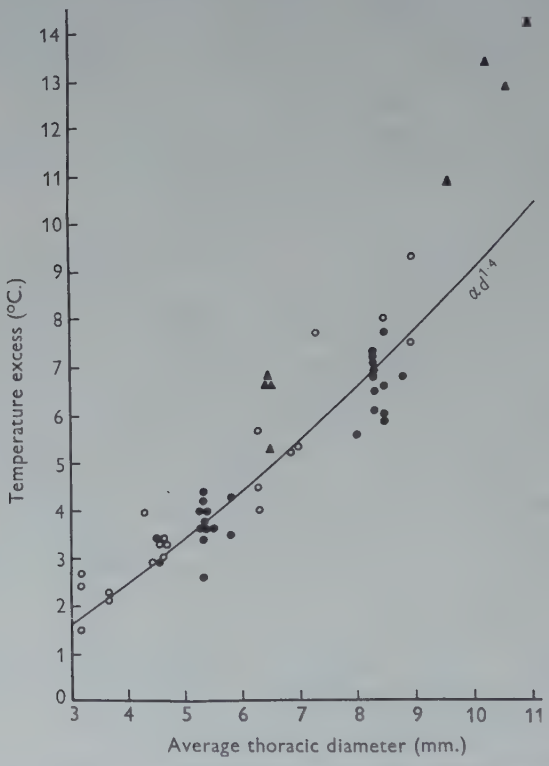
The relationships between the factors governing convective heat loss have been well worked out for regularly shaped bodies, especially long cylinders. By *forced* convection (as in flight or in a wind) the rate of heat loss, $H_f$, per unit surface area is determined by a dimensionless equation of the form

$$\frac{H_f d}{k\theta} = C\left(\frac{\rho v d}{\mu}\right)^x \left(\frac{c\mu}{k}\right)^y,$$

where $d$ is the diameter of the body (or some other characteristic linear dimension), $\theta$ is the difference in temperature between the surface and the oncoming fluid, $k$ is the conductivity of the fluid, $\rho$ is the density, $v$ the velocity, $\mu$ the viscosity, and $c$ the specific heat of the fluid. $C$, $x$ and $y$ are constants, derived experimentally and dependent mainly on the shape of the body and whether the fluid is in laminar or turbulent flow. $\rho vd/\mu$ is the Reynolds number, $Re$; $Hd/k\theta$ the Nusselt number, $Nu$; and $c\mu/k$ the Prandtl number.

The values of $k$, $\rho$, $\mu$ and $c$ do not vary much for air under ordinary conditions so the equation can be considerably simplified. According to Fishenden & Saunders (1950), for forced convection of air *across* a cylinder $Nu \approx 0{\cdot}24Re^{0{\cdot}6}$ if the logarithm of $Re$ is between 3 and 5. In my examples, however, log $Re$ is at the lower end of this range and $Nu \approx 0{\cdot}45Re^{0{\cdot}5}$ should be more appropriate. (The

constants were evaluated from Fishenden & Saunders's (1950) Fig. 37, p. 129, according to the authors' procedure.) It would not be surprising, though, if the irregular shape of an insect's body altered the relationship to some extent. Any increase in the turbulence of the air flow caused by irregularities in the surface would have an effect similar to an increase in the Reynolds number. *Nu* then might be more nearly proportional to $Re^{0.6}$ after all, or even to a higher power.




Text-fig. 1. Relationship between size of pterothoracic capsule and the temperature excess in artificially heated, uninsulated insects in a 300 cm./sec. wind. Heating was proportional to the volume of the pterothorax. An insect with a 10 mm. average-diameter pterothorax was given 2·67 cal./min. Black circles, denuded bumble-bees; white circles, denuded moths; triangles, dragonflies with air sacs filled with water.

In (some) other instances—where the body is not a cylinder across the air stream—the relationships between *Nu* and *Re* are essentially the same, differing mainly in the appropriate values for *C*. If the axis of the cylinder is *parallel* to the wind direction the rate of cooling per square centimetre is half, and if the body is a *sphere* with the same diameter it is about one and one-half times the rate for a cylinder perpendicular to the wind.

The pterothoracic capsule of a bee, moth, or dragonfly most resembles the sphere, except that in a wind it is partly shielded front and back by the head and prothorax and the abdomen. The whole insect, on the other hand, is perhaps more like the cylinder, parallel to the air stream when in flight, and the pterothorax

may be considered a section of it; but the air flow over it is probably rather turbulent because of the deep constrictions between the tagmata and the rate of cooling is probably higher than for a smooth cylinder.

Part of the heat loss from the insects, however, was due to *natural* convection produced by the buoyancy of the warmer air around the warm body. An idea of the amount of cooling due to natural convection can be obtained by calculating the equivalent heat loss from a horizontal cylinder. In this case $Nu$ is a function of the Prandtl number, $Pr$, and the Grashof number, $Gr$. $Gr = ag\theta d^3\rho^2/\mu^2$, where $a$ is the coefficient of expansion of the fluid, $g$ is the acceleration due to gravity, and the other letters are the same as before. Typically $Nu = 0{\cdot}91\ (GrPr)^{0{\cdot}17}$ when $\log(GrPr)$ is between 1 and 3, as it is in our examples. (The constants were estimated from Fishenden & Saunders' (1950) Fig. 24, p. 91.) The equation can be reduced to $H_n = 1{\cdot}24 \times 10^{-4}(\theta^{1{\cdot}17}/d^{0{\cdot}49})$ cal./sec. cm.$^2$ if suitable values are taken for the density, viscosity, etc., which ordinarily vary over only a small range.

Now, how do the rates of cooling of the insects compare with those of regular cylinders and spheres? As an example let us take an imaginary large denuded 'moth-bumble-bee' with an average pterothoracic diameter of 8·5 mm. and a heat input of 1·64 cal./min. Judged from Text-fig. 1, its temperature excess typically would be about 7·2 or 7·3° C. in a 300 cm./sec. wind. The temperature excess at the surface of the pterothorax would be about 6·5° C. Of the total heat input something like 1·30 cal./min., or 0·022 cal./sec., would be dissipated from the pterothorax by convection. (This includes the heat removed directly by both forced and natural convection but not that first conducted to other parts of the body.) If the surface of the pterothoracic capsule was 2·25 cm.$^2$ the convective heat loss would be 0·010 cal./sec. cm.$^2$.

Natural convection in still air from a horizontal cylinder the same diameter and temperature would amount to a little over 0·001 cal./sec. cm.$^2$, equivalent to only 10–15 % of the convective heat loss from the 'bee-moth'. (Natural convection might be less in a wind, depending on the extent to which natural and forced convection are additive when calculated this way.) Forced convection must be responsible for the 0·009 cal./sec. cm.$^2$, or so, remaining.

The rate of heat loss by forced convection from a smooth cylinder with the same diameter as the insect's thorax, with the same temperature excess, and parallel to the same wind, would equal $(0{\cdot}45k\theta/2d)\ (\rho vd/\mu)^{0{\cdot}5}$. If $k$ is $6{\cdot}2 \times 10^{-5}$ and $\rho/\mu$ is 6·9, $H_f = 0{\cdot}004$ or 0·005 cal./sec. cm.$^2$. From a sphere the same diameter $H_f$ would be about 3 times as great—0·012–0·015 cal./sec. cm.$^2$. The insect's 0·009 cal./sec. cm.$^2$ falls between the two, as might have been predicted.

The hypothetical 'moth-bumble-bee' was a rather large insect. How much faster do smaller ones cool? Is the relationship between their size and the rate of convection the same as for equivalent cylinders and spheres?

From the forced convection equation, $(H_f d/k\theta) = C(\rho vd/\mu)^{0{\cdot}5}$, one can extract $(H_f/\theta) \propto (v^{0{\cdot}5}/d^{0{\cdot}5})$. Then $H_f A/\theta \propto v^{0{\cdot}5}d^{1{\cdot}5}$, if $A$ is the surface area of the body and is proportional to $d^2$, and $H_f A$ is the total rate of heat loss. But at equilibrium $H_f A$ must be equal to the heat input, which was set proportional to $d^3$. Therefore

$\theta \propto (d^{1.5}/v^{0.5})$. (If $Nu$ is actually proportional to $Re^{0.6}$ rather than $Re^{0.5}$, then $\theta$ will be proportional to $d^{1.4}/v^{0.6}$, etc.)

In the dragonflies and denuded bumble-bees and moths in Text-fig. 1 the temperature excess generally was proportional to the 1·3–1·5 power of the average diameter of the pterothoracic capsule. (A line proportional to $d^{1.4}$ and lying among the bees and moths has been superimposed on the figure for comparison.) Perhaps the average temperature excess would have followed closer to $d^{1.3}$ if the large and small insects had lost exactly the same fraction of their heat by convection from the thorax—in the small insects probably a little more escaped by other routes than in the larger ones. At any rate, the general relationship between size and temperature excess was not very much different from that for perfect cylinders and spheres. The additional turbulence in the air flow over the insects that has already been suggested may account for the small difference.

Natural convection can be ignored in this context. As we have seen, it would have contributed only a little cooling compared with the 300 cm./sec. wind, and within the pertinent range of dimensions and temperatures the relationship between $\theta$ and $d$ is roughly the same in either case.

Digby (1955) reported that convective cooling agreed well with theory in long, narrow insects and in plasticine spheres warmed by radiation from a lamp. However, in small, short-bodied Diptera and Hymenoptera the temperature excess increased much faster with size than expected. He suggested that the effect was 'due to change in absorptivity or the position of absorptivity (perhaps both) with size'. The fact that my results with internally heated specimens were quite different supports his explanation; insects are partly transparent and probably a considerable part of the radiant energy in Digby's experiments passed through the smaller specimens and was not absorbed.

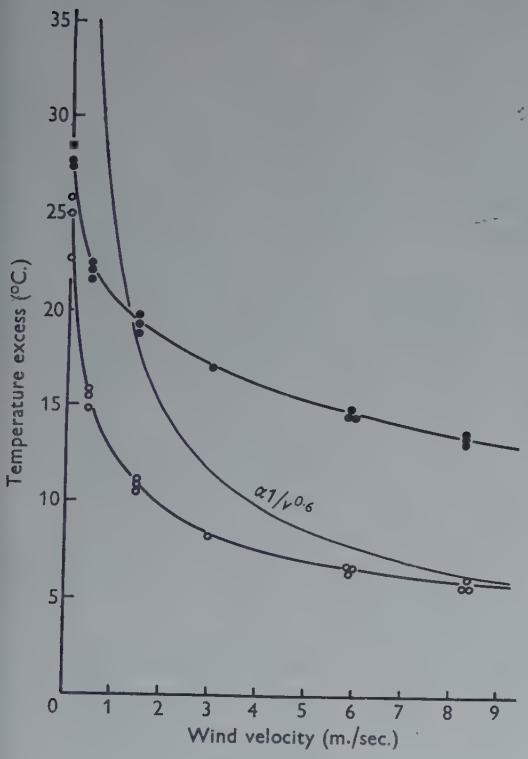
*Effects of air speed on cooling of naked and insulated insects*

Three questions remain. How is convection from a naked insect related to the wind velocity; is the relationship the same as for a smooth, regular object? How does the wind speed affect the insect's temperature excess? Finally, how well does an insect's hair insulate it in winds stronger or lighter than 300 cm./sec.? The first two questions can best be answered together.

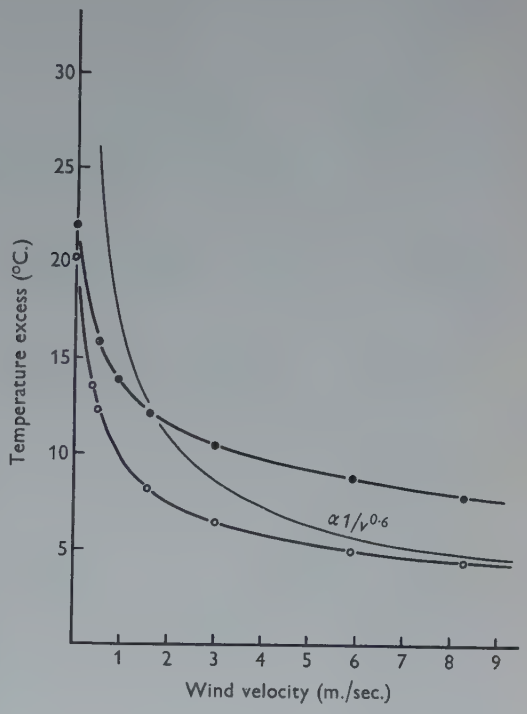
The three *Sphinx ligustri* and several of the *Bombus terrestris* and *lapidarius* queens already referred to were used as examples. They were subjected to winds from 0 to 830 cm./sec. with their hair and legs intact and with these removed. The results are summarized in Text-figs. 2 and 3. To show the effects of air speed more clearly the original temperature excess curves were raised or lowered slightly so that in each group the values at 300 cm./sec. would coincide with their mean. The points on any one curve all were adjusted by the same fraction.

In the denuded bumble-bees and moths, if $\theta$ is proportional to $d^{1.4}$, or thereabouts, it should also be proportional to $1/v^{0.6}$—or it should be if all the heat were dissipated by the wind. In the slowest winds, where most of the cooling was by natural convection and radiation, the temperature excess of naked *Sphinx* and *Bombus*

departed widely from this relationship (Text-fig. 2 and 3, lower curves), as one would expect. Above 300 cm./sec. the deviations were still fairly large; between 300 and 800 or 900 cm./sec. the temperature excess actually was proportional to $1/v^{0.3}$ in *Sphinx* and $1/v^{0.4}$ *in Bombus*.




Text-fig. 2




Text-fig. 3

Text-fig. 2. Effects of wind velocity on the temperature excess in normal and denuded *Sphinx ligustri*, artificially heated. Average diameter of pterothoracic capsules was 8·8 mm. Black circles, hair and legs intact; white circles, hair and legs removed.

Text-fig. 3. Effects of wind velocity on the average temperature excess in normal and denuded queen bumble-bees. Average diameter of pterothoracic capsules was 8·4 mm. Black circles, hair and legs intact; white circles, hair and legs removed.

Although it was probably acceptable to equate $\theta$ with the internal temperature excess when relating temperature excess to size, $\theta$ is properly the temperature excess at the surface of the pterothorax, which was probably about three-quarters of a degree lower in each case than the values recorded in Text-figs. 2 and 3. Substituting surface temperature excesses for those of the interior would bend the curves a little closer to the ideal shape.

But the main reason for the differences between the experimental and ideal curves lies in the increase in the relative amount of heat dissipated by forced convection as the wind is increased. Take as an example a denuded bumble-bee with an average pterothoracic diameter of 8·4 mm. and surface of about 2·2 cm.$^2$. If the

total heat loss is 1·56 cal./min. the rate of cooling will be 0·012 cal./sec. cm.$^2$. In a 300 cm./sec. wind long-wave radiation will account for about 0·0006 cal./sec. cm.$^2$, or 5 % of the total, and evaporation a small amount. Probably 10 or 15 % of the heat will be conducted to other parts of the body and the thermojunction and electrode wires. The rest of the cooling will be by convection. If natural convection takes 0·001 cal./sec. cm.$^2$, not quite 10 % of the total, then 70–75 % will remain to the wind.

In the laboratory the total heat loss by natural convection, radiation, evaporation, and conduction increases or decreases roughly in proportion to the surface temperature excess, if the air speed is the only independent variable. Although natural convection and radiation change a little faster than the temperature excess, conduction to the abdomen, etc., lags behind. At 300 cm./sec. the temperature excess of the bumble-bee would be about 6·5° C. (Text-fig. 3), which would indicate about 5·8° C. at the surface. A 900 cm./sec. wind would reduce the internal temperature excess to about 4·3° C. and the surface to 3·6° C. Cooling by means other than forced convection would thereby be reduced to 15–20 % of the total. The wind then would have to account for 80 or 85 % of the heat instead of 70–75 %, a relative increase of about 15 %.

If the bumble-bees in Text-fig. 3 had been given 15 % more heat at 300 cm./sec. than at 900, the average *surface* temperature excess would have been very nearly proportional to $1/v^{0·6}$.

If the denuded sphinx moths (Text-fig. 2) were treated similarly the average surface temperature excess would be proportional to $1/v^{0·5}$ between 300 and 900 cm./sec., rather than $1/v^{0·6}$, but that is reasonably close.

Furthermore, in *Bombus*, if 25–30 % of the cooling is by mechanisms other than forced convection when the surface temperature excess is 5·8° C., it should reach 100 % between about 19 and 23° C. And in *Sphinx*, if it is 25–30 % at 7·5° C., it should reach 100 % at 25–30° C. In other words, these should be the equilibrium temperature excesses when there is no wind. In fact, the average surface temperature excess was 19–20° C. in *Bombus* and 23–24° C. in *Sphinx* when the wind was turned off and the insects were shielded fairly closely against stray air currents.

Finally, let us compare the intact insects represented in Text-figs. 2 and 3 with the denuded ones. Two points of particular interest are evident. First, the hair made little difference to the insects' temperatures in still air. And second, the (absolute) temperature increases produced by the insulation were nearly constant over the whole range between 200 and 800 or 900 cm./sec. The temperature increases did decline, as they must, after reaching maximum values at about 200 cm./sec., but they declined very slowly—and the *relative* increases in the temperature excess got larger.

Part of the explanation is obvious: more heat is lost by radiation when the air is still or nearly still, and radiation is not much affected by the presence of hair. But that cannot be the whole answer, for more heat is lost by natural convection than by radiation. The hair must offer relatively little resistence to the gentle air movement of natural convection and much more resistance to a fast stream.

A stationary insect exercising (or sunning) in a sheltered spot would get but little help from its fur.* It would hardly need it, for cooling would be much slower than in flight anyway. In flight, however, a good coat of fur greatly reduces convection; furthermore, its effect is scarcely smaller at 800 or 900 cm./sec. than at 200 or 300, and few insects fly faster than that.

## SUMMARY

1. The natural internal temperature gradients during flight were reproduced in various medium and large insects by mounting freshly killed specimens in a wind tunnel and heating them with a high-frequency electric current. The heat flow from the flight muscles to other parts of the body and from the body were investigated.

2. Comparison of dead and living insects showed that most of the heat transfer within the body is by conduction; circulation of the haemolymph during flight contributes little to the heat flow.

3. The temperature excess is high throughout the pterothorax in a large insect; where there are no subcutaneous air sacs it is only about 10 % less at the surface of the pterothorax than at the centre.

4. Only about 5–15 % of the heat generated in the flight muscles is conducted to the prothorax, head, abdomen and appendages, which remain near the temperature of the air.

5. Usually not more than 10–15 % of the heat escapes from the pterothorax by long-wave radiation in a large insect flying under a clear sky. Smaller insects lose relatively more of their heat by radiation.

6. Radiation increases with the insect's temperature but it is never sufficient to give much protection against overheating.

7. Ordinarily 60–80 % of the heat is dissipated from the surface of the pterothorax by convection.

8. In convection from a naked insect the relationships between heat loss, the surface temperature excess, size, and wind speed are nearly the same as in convection from a smooth cylinder or sphere, if allowance is made for turbulence in the air flow over the insect.

9. In dragonflies and denuded bees and moths heated in proportion to their pterothoracic volumes in a constant wind, the temperature excess was proportional to the 1·3–1·5 power of the average diameter of the pterothorax.

10. The coats of hair on bumble-bees, hawk moths, and noctuid moths are excellent insulators against convective heat loss. At normal flying speeds they increase the temperature excess by 50–100 % or more—in a large hawk moth probably by at least 8 or 9° C.

11. The insulating value of a coat depends mostly on its density and on the size of the insect, and less on the length of the hair.

* Probably the main reason Digby (1955) found no difference between pubescent and hairless insects irradiated in a wind was that the wind was only 50 cm./sec. However, Weis-Fogh (personal communication) has suggested that the hair may have interfered with penetration of the radiant energy.

12. In dragonflies the pterothorax is insulated nearly as effectively by the subcutaneous air sacs.

I am most grateful to Prof. V. B. Wigglesworth, F.R.S., Dr R. W. Salt, and many others in the Department of Zoology, University of Cambridge, and the Research Branch, Canada Department of Agriculture, for their support and encouragement; to Dr T. Weis-Fogh for lending me his high-frequency generator; to Dr F. S. J. Hollick for the use of his device for calibrating hot-wire anemometers; and to Mr J. Weintraub, Dr W. C. Broadfoot, and Dr Salt of the Lethbridge Research Station for reading the manuscript.

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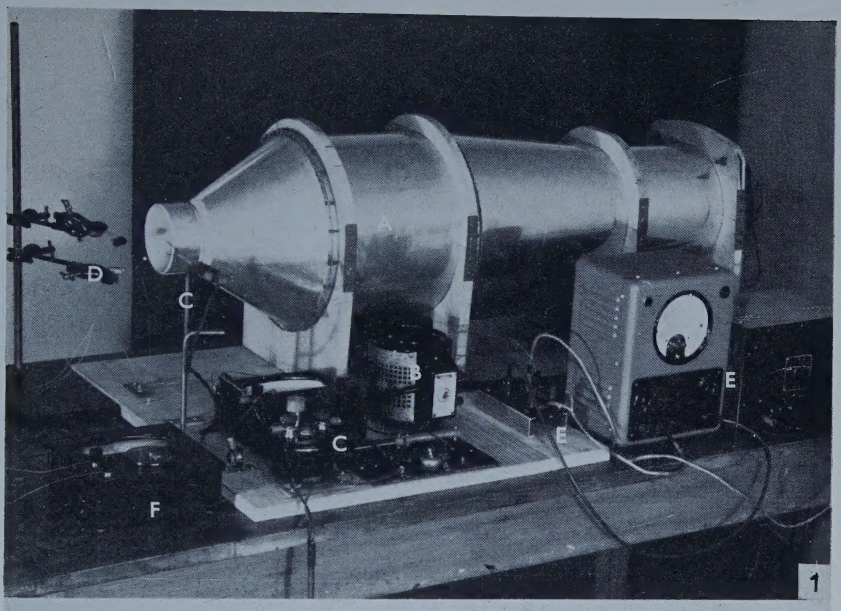
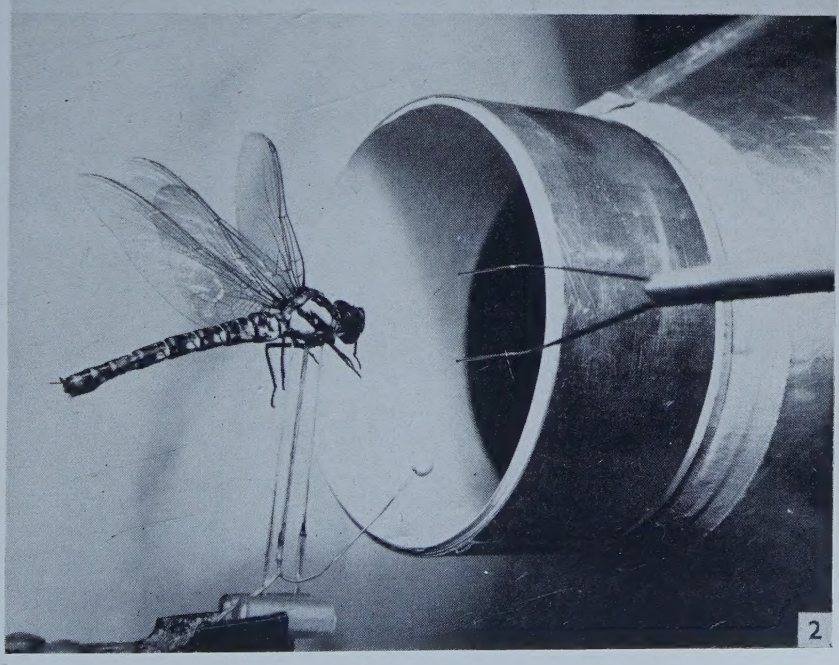
## EXPLANATION OF PLATE

Fig. 1. Wind tunnel and heating and measuring apparatus. A, wind tunnel; B, variable transformer for wind tunnel fan; C, hot-wire anemometer assembly; D, bumble-bee supported in air stream by electrode and thermojunction holders; E, heating assembly (switchboard, voltmeter, high-frequency generator); F, ammeter for thermocouple.

Fig. 2. Female *Aeschna cyanea* mounted in air stream. Hot wire of anemometer at right.




CHURCH—HEAT LOSS AND THE BODY TEMPERATURES OF FLYING INSECTS. II

(*Facing p.* 212)

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